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**WO 02/04489 A2**

(54) Title: **POLYMORPHIC KINASE ANCHOR PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME**

(57) Abstract: Polymorphic A-Kinase anchor proteins (AKAPs) and nucleic acids encoding the proteins are provided herein. Methods of detecting polymorphic AKAPs and nucleic acids encoding the AKAPs, and kits for use in the detection methods are also provided. Further provided herein are methods of identifying subjects having or at risk of developing disorders of signal transduction. Methods of determining susceptibility to morbidity and/or increased or early mortality are also provided.

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**POLYMORPHIC KINASE ANCHOR PROTEINS AND  
NUCLEIC ACIDS ENCODING THE SAME**

**RELATED APPLICATIONS**

Benefit of priority is claimed to U.S. application Serial No. 09/834,700, filed  
5 April 12, 2001, to U.S. provisional application Serial No. 60/240,335, filed  
October 13, 2000, to Andreas Braun, entitled "POLYMORPHIC KINASE  
ANCHOR PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME" and to U.S.  
provisional application Serial No. 60/217,251, filed July 10, 2000, to Andreas  
Braun, entitled "POLYMORPHIC KINASE ANCHOR PROTEINS AND NUCLEIC  
10 ACIDS ENCODING THE SAME" is claimed herein.

For U.S. purposes this application is a continuation of U.S. application  
Serial No. 09/834,700. Benefit of priority under 35 U.S.C. §119(e) to U.S.  
provisional application Serial No. 60/217,251, filed July 10, 2000, to Andreas  
Braun, entitled "POLYMORPHIC KINASE ANCHOR PROTEINS AND NUCLEIC  
15 ACIDS ENCODING THE SAME" is claimed herein. Benefit of priority under  
35 U.S.C. §119(e) to U.S. provisional application Serial No. 60/240,335, filed  
October 13, 2000, to Andreas Braun, entitled "POLYMORPHIC KINASE  
ANCHOR PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME" also is  
claimed herein.

20 This application is related to U.S. Application Serial No. 09/687,483 and  
International PCT Application No. PCT/US00/28413, each to Andreas Braun,  
Hubert Köster and Dirk van den Boom, each entitled "METHODS FOR  
GENERATING DATABASES AND DATABASES FOR IDENTIFYING  
POLYMORPHIC GENETIC MARKERS", and each filed October 13, 2000. This  
25 application is also related to U.S. Provisional Application Serial No. 60/159,176  
to Andreas Braun, Hubert Köster; Dirk Van den Boom, filed October 13, 1999,  
entitled "METHODS FOR GENERATING DATABASES AND DATABASES FOR  
IDENTIFYING POLYMORPHIC GENETIC MARKERS". This application is also  
related to U.S. Provisional Application Serial No. 60/217,658 to Andreas Braun,  
30 Hubert Köster; Dirk Van den Boom, filed July 10, 2000, entitled "METHODS  
FOR GENERATING DATABASES AND DATABASES FOR IDENTIFYING  
POLYMORPHIC GENETIC MARKERS".



Where permitted, the subject matter of each of above-noted U.S. provisional applications, U.S. applications and PCT application is incorporated herein in its entirety.

#### FIELD OF THE INVENTION

- 5 Polymorphic A-kinase anchor proteins (AKAPs) and nucleic acids encoding the proteins are provided herein. Methods of detecting polymorphic AKAPs and nucleic acids encoding the AKAPs, and kits for use in the detection methods are also provided. Further provided herein are methods of identifying subjects having or at a risk of developing disorders of cellular protein
- 10 phosphorylation and/or signal transduction. Methods of determining susceptibility to morbidity and/or increased or early mortality are also provided.

#### BACKGROUND OF THE INVENTION

- Protein phosphorylation is an important mechanism for enzyme regulation and the transduction of extracellular signals across the cell membrane in
- 15 eukaryotic cells. A wide variety of cellular substrates, including enzymes, membrane receptors, ion channels and transcription factors, can be phosphorylated in response to extracellular signals that interact with cells. A key enzyme in the phosphorylation of cellular proteins in response to hormones and neurotransmitters is cyclic AMP (cAMP)-dependent protein kinase (PKA).
- 20 Upon activation by cAMP, PKA thus mediates a variety of cellular responses to such extracellular signals. An array of PKA isozymes are expressed in mammalian cells. The PKAs usually exist as inactive tetrameres containing a regulatory (R) subunit dimer and two catalytic (C) subunits. Genes encoding three C subunits ( $C\alpha$ ,  $C\beta$  and  $C\gamma$ ) and four R subunits ( $R1\alpha$ ,  $R1\beta$ ,  $R11\alpha$  and  $R11\beta$ )
- 25 have been identified (see Takio *et al.* (1982) *Proc. Natl. Acad. Sci. U.S. A.* 79:2544-2548; Lee *et al.* (1983) *Proc. Natl. Acad. Sci. U.S. A.* 80:3608-3612; Jahnsen *et al.* (1996) *J. Biol. Chem.* 261:12352-12361; Clegg *et al.* (1988) *Proc. Natl. Acad. Sci. U.S. A.* 85:3703-3707; and Scott (1991) *Pharmacol. Ther.* 50:123-145). The type I (RI)  $\alpha$  and type II (RII)  $\alpha$  subunits are distributed
- 30 ubiquitously, whereas  $R1\beta$  and  $R11\beta$  are present mainly in brain (see. *e.g.*, Miki and Eddy (1999) *J. Biol. Chem.* 274:29057-29062). The type I PKA holoenzyme ( $R1\alpha$  and  $R1\beta$ ) is predominantly cytoplasmic, whereas the majority of

type II PKA (RI $\alpha$  and RI $\beta$ ) associates with cellular structures and organelles (Scott (1991) *Pharmacol. Ther.* 50:123-145). Many hormones and other signals act through receptors to generate cAMP which binds to the R subunits of PKA and releases and activates the C subunits to phosphorylate proteins.

- 5           Because protein kinases and their substrates are widely distributed throughout cells, there are mechanisms in place in cells to localize protein kinase-mediated responses to different signals. One such mechanism involves subcellular targeting of PKAs through association with anchoring proteins, referred to as A-kinase anchoring proteins (AKAPs), that place PKAs in close
- 10           proximity to specific organelles or cytoskeletal components and particular substrates thereby providing for more specific PKA interactions and localized responses (see, *e.g.*, Scott *et al.* (1990) *J. Biol. Chem.* 265:21561-21566; Bregman *et al.* (1991) *J. Biol. Chem.* 266:7207-7213; and Miki and Eddy (1999) *J. Biol. Chem.* 274:29057-29062). Anchoring not only places the kinase close
- 15           to preferred substrates, but also positions the PKA holoenzyme at sites where it can optimally respond to fluctuations in the second messenger cAMP (Mochly-Rosen (1995) *Science* 268:247-251; Faux and Scott (1996) *Trends Biochem. Sci.* 21:312-315; Hubbard and Cohen (1993) *Trends Biochem. Sci.* 18:172-177).
- 20           Up to 75% of type II PKA is localized to various intracellular sites through association of the regulatory subunit (RII) with AKAPs (see, *e.g.*, Hausken *et al.* (1996) *J. Biol. Chem.* 271:29016-29022). RII subunits of PKA bind to AKAPs with nanomolar affinity (Carr *et al.* (1992) *J. Biol. Chem.* 267:13376-13382), and many AKAP-RII complexes have been isolated from cell extracts. RI
- 25           subunits of PKA bind to AKAPs with only micromolar affinity (Burton *et al.* (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94:11067-11072). Evidence of binding of a PKA RI subunit to an AKAP has been reported (Miki and Eddy (1998) *J. Biol. Chem.* 273:34384-34390) in which RI $\alpha$ -specific and RI $\alpha$ /RII $\alpha$  dual specificity PKA anchoring domains were identified on FSC1/AKAP82. Additional dual specific
- 30           AKAPs, referred to as D-AKAP1 and D-AKAP2, which interact with the type I and type II regulatory subunits of PKA have also been reported (Huang *et al.*

(1997) *J. Biol. Chem.* 272:8057-8064; Huang *et al.* (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94:11184-11189).

More than 20 AKAPs have been reported in different tissues and species. Complementary DNA (cDNA) encoding AKAPs have been isolated from diverse species, ranging from *Caenorhabditis elegans* and *Drosophila* to human (see, 5 *e.g.*, Colledge and Scott (1999) *Trends Cell Biol.* 9:216-221). Regions within AKAPs that mediate association with RII subunits of PKA have been identified. These regions of approximately 10-18 amino acid residues vary substantially in primary sequence, but secondary structure predictions indicate that they are 10 likely to form an amphipathic helix with hydrophobic residues aligned along one face of the helix and charged residues along the other (Carr *et al.* (1991) *J. Biol. Chem.* 266:14188-14192; Carr *et al.* (1992) *J. Biol. Chem.* 267:13376-13382). Hydrophobic amino acids with a long aliphatic side chain, *e.g.*, valine, leucine or isoleucine, may participate in binding to RII subunits (Glantz *et al.* (1993) *J. Biol.* 15 *Chem.* 268:12796-12804).

Many AKAPs also have the ability to bind to multiple proteins, including other signalling enzymes. For example, AKAP79 binds to PKA, protein kinase C (PKC) and the protein phosphatase calcineurin (PP2B) (Coghlan *et al.* (1995) *Science* 267:108-112 and Klauck *et al.* (1996) *Science* 271:1589-1592). 20 Therefore, the targeting of AKAP79 to neuronal postsynaptic membranes brings together enzymes with opposite catalytic activities in a single complex.

AKAPs thus serve as potential regulatory mechanisms that increase the selectivity and intensity of a cAMP-mediated response. There is a need, therefore, to identify and elucidate the structural and functional properties of 25 AKAPs in order to gain a complete understanding of the important role these proteins play in the basic functioning of cells.

#### SUMMARY OF THE INVENTION

Previously unidentified alleles of the human AKAP10 gene are provided. One allele, designated AKAP10-5 contains a previously undisclosed single 30 nucleotide polymorphism (SNP), an A-to-G transition, at nucleotide position 2073 of the AKAP10 gene coding sequence. This SNP is located in the C-terminal PKA binding domain, and results in an Ile-to-Val substitution at the protein level

for the AKAP10 gene protein product. Another allele, designated AKAP10-6 contains a previously undisclosed single nucleotide polymorphism (SNP), a C-to-G transversion, at nucleotide position 83587 of the human chromosome 17 sequence (SEQ ID NO: 17). The AKAP10 gene is located at approximately  
5 nucleotide position 83,580 to nucleotide position 156,577 of the chromosome 17 sequence. This SNP is located in the 5' untranslated region and 132 nucleotides upstream of the translation start site. A further allele, designated AKAP10-7 contains a previously undisclosed single nucleotide polymorphism (SNP), a G-to-A transition, at nucleotide position 129,600 of the human  
10 chromosome 17 sequence. This SNP is located four bases 3' to the exon 10/intron 10 boundary of AKAP10 mRNA.

Using a healthy patient database, the frequency of occurrence of two allelic variants of the AKAP10 gene, AKAP10-5 and AKAP10-1, in such a population were found to decrease with age. AKAP10-1 is an allelic variant with  
15 a T to C transversion at nucleotide position 156,277 of the AKAP10 genomic clone which is located in the 3' untranslated region of the gene. The AKAP10-5 and AKAP10-1 alleles are useful markers for predicting susceptibility to morbidity and/or increased or early mortality. Methods are provided for predicting susceptibility to morbidity, increased or early mortality, or morbidity and  
20 increased mortality, by detecting the presence of the AKAP10 allelic variants, individually, or in combination with other AKAP10 allelic variants, in an organism, particularly an animal and particularly a human. Methods are also provided for indicating or predicting an alteration in signal transduction in an organism. Furthermore, AKAP10-5 and other allelic variants of the AKAP10  
25 gene are potential functional variants of a morbidity susceptibility gene and/or of a gene involved in increased mortality and/or a gene related to an alteration in signal transduction and associated disorders and thus may also be useful for screening for potential therapeutics.

**AKAP10-5 nucleic acids and proteins**

The nucleotide sequence of the coding sequence of the predominant allele (i.e., the allele present in the greatest frequency for a given population) of the AKAP-10 gene is presented in SEQ ID NO: 1 and the encoded protein sequence  
5 in SEQ ID NO: 2. The nucleotide sequence of the coding sequence of the allelic variant AKAP10-5 is presented as SEQ ID NO: 3 and the corresponding protein sequence as SEQ ID NO: 4.

Also provided is an isolated nucleic acid molecule comprising a sequence of nucleotides that encodes the polypeptide as set forth in SEQ ID NO: 2, except  
10 that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with Val, Leu or Phe.

Further provided is an isolated nucleic acid molecule comprising a sequence of nucleotides that encodes the polypeptide as set forth in SEQ ID NO: 2, except that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with  
15 Val.

Also provided is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as position 138 to position 2126 of SEQ ID NO: 1, except that the nucleotide at position 2073 of SEQ ID NO: 1 is replaced with a nucleotide selected from among G, T and C.

20 Further provided is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as position 138 to position 2126 of SEQ ID NO: 1, except that the nucleotide at position 2073 of SEQ ID NO: 1 is replaced with a G nucleotide.

Further provided is an isolated nucleic acid molecule, comprising a  
25 sequence of nucleotides that encodes the polypeptide of SEQ ID NO: 4.

Also provided is an isolated nucleic acid molecule comprising nucleotides from position 138 to position 2126 of SEQ ID NO: 3.

Further provided is an isolated nucleic acid molecule, comprising at least  
14 or 16 contiguous nucleotides of SEQ ID NO: 3; wherein the contiguous  
30 nucleotides include a sequence of 5 contiguous nucleotides as set forth from position 2069 to position 2077 of SEQ ID NO: 3.

Also provided is an isolated nucleic acid molecule, comprising at least 30 contiguous nucleotides of SEQ ID NO 3; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides as set forth from position 2069 to position 2077 of SEQ ID NO: 3.

- 5        Also provided is an isolated nucleic acid molecule, comprising at least 50 contiguous nucleotides of SEQ ID NO 3; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides as set forth from position 2069 to position 2077 of SEQ ID NO: 3.

- 10       Further provided is a polypeptide comprising the sequence of amino acid residues as set forth in SEQ ID NO 2, except that the Ile residue at position 646 of SEQ ID NO 2 is replaced with Val, Leu or Phe.

Also provided is a polypeptide comprising the sequence of amino acid residues as set forth in SEQ ID NO 4.

- 15       Further provided is a portion of the polypeptide encoded by the nucleic acid molecule comprising a sequence of nucleotides that encodes the polypeptide as set forth in SEQ ID NO: 2, except that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with Val, Leu or Phe and further comprising at least 5 or 6 amino acid residues and including the replaced residue at position 646 of SEQ ID NO: 2.

- 20       Also provided is the polypeptide as described above, wherein the residue at position 646 of SEQ ID NO: 2 is Val.

- 25       Further provided are primers, probes or antisense nucleic acid molecules that specifically hybridize adjacent to or at a polymorphic region spanning a position corresponding to position 2073 of SEQ ID NO 1 or 3 of an AKAP10 allele or the complement thereof.

Also provided are such primers, probes or antisense nucleic acid molecules as described above that hybridize under moderate or high conditions.

- 30       Further provided is a primer that specifically hybridizes at a position immediately adjacent to a position corresponding to position 2073 of SEQ ID NO: 1 or 3 of an AKAP10 allele.

Also provided is a primer that is extended by a nucleotide that specifically base pairs with the nucleotide at a position corresponding to position 2073 of SEQ ID NO: 3 of an AKAP10 allele.

Further provided are such primers, probes or antisense nucleic acid molecules as described above, that are single-stranded and comprise at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the sequence of nucleotides includes at least 5 contiguous nucleotides from position 2069 to position 2077 of SEQ ID NO: 3. These primers, probes or antisense nucleic acid molecules can also comprise at least 20 or 30 contiguous nucleotides of SEQ ID NO: 3, or the complement thereof.

Further provided are such primers, probes or antisense nucleic acid molecules as described above, that are single-stranded and comprise at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5 contiguous nucleotides from position 2069 to position 2077 of SEQ ID NO: 1, except that the nucleotide at position 2073 of SEQ ID NO: 1 is replaced with a nucleotide selected from among G, T and C.

Further provided are nucleic acid vectors comprising nucleic acid molecules as described above, including a nucleic acid molecule comprising a sequence of nucleotides that encodes the polypeptide as set forth in SEQ ID NO: 2, except that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with Val, Leu or Phe.

Also provided is a primer that has the sequence set forth in SEQ ID NO: 15.

Also provided are cells containing the nucleic acid vectors described above.

#### **AKAP10-6 nucleic acids**

The nucleotide sequence of chromosome 17 containing the genomic sequence of the predominant allele (i.e., the allele present in the greatest frequency for a given population) of the AKAP-10 gene is set forth in SEQ ID NO: 17. The nucleotide sequence of chromosome 17 containing the genomic sequence of the allelic variant AKAP10-6 is presented as SEQ ID NO: 13.

Provided herein is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as nucleotides of position 83,580 to position 156,577 of SEQ ID NO: 17, except that the nucleotide at position 83587 of SEQ ID NO: 17 is replaced with a nucleotide selected from among G, A and T.

- 5 Further provided is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as nucleotides of position 83,580 to position 156,577 of SEQ ID NO: 17, except that the nucleotide at position 83587 of SEQ ID NO: 17 is replaced with the nucleotide C.

- Also provided is an isolated nucleic acid molecule comprising the  
10 sequence of nucleotides set forth as nucleotides of position 83,580 to position 156,577 of SEQ ID NO: 13.

- Further provided is an isolated nucleic acid molecule, comprising at least 14 or 16 contiguous nucleotides of SEQ ID NO: 13; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides from position 83583  
15 to position 83591 of SEQ ID NO: 13.

Also provided is an isolated nucleic acid molecule, comprising at least 30 contiguous nucleotides of SEQ ID NO: 13; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides from position 83583 to position 83591 of SEQ ID NO: 13.

- 20 Also provided is an isolated nucleic acid molecule, comprising at least 50 contiguous nucleotides of SEQ ID NO: 13; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides from position 83583 to position 83591 of SEQ ID NO: 13.

- Further provided are primers that specifically hybridize at a position  
25 immediately adjacent to a position corresponding to position 83587 of SEQ ID NO: 13 or 17 of an AKAP10 allele.

- Further provided are primers, probes and antisense nucleic acid molecules that specifically hybridize adjacent to or at a polymorphic region spanning a position corresponding to position 83587 of SEQ ID NO: 13 or 17 of an AKAP10  
30 allele or the complement thereof.

Also provided are such primers, probes or antisense nucleic acid molecules as described above that hybridize under moderate or high conditions.



Further provided are primers that specifically hybridize at a position immediately adjacent to a position corresponding to position 83587 of SEQ ID NO: 13 or 17 of an AKAP10 allele.

Also provided are primers that are extended by a nucleotide that  
5 specifically base pairs with the nucleotide at a position corresponding to position 83587 of SEQ ID NO: 13 of an AKAP10 allele.

Further provided are such primers, probes or antisense nucleic acid molecules as described above, that are single-stranded and comprise at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof,  
10 wherein the nucleic acid molecule includes at least 5 contiguous nucleotides from position 83583 to position 83591 of SEQ ID NO: 13. The primers, probes or antisense nucleic acid molecules can also comprise at least 20 or 30 contiguous nucleotides of SEQ ID NO: 13, or the complement thereof.

Further provided are such primers, probes or antisense nucleic acid  
15 molecules as described above, that are single-stranded and comprise at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecules include at least 5 contiguous nucleotides from position 83583 to position 83591 of SEQ ID NO: 17, except that the nucleotide at position 83587 of SEQ ID NO 17 is replaced with a nucleotide  
20 selected from among G, A and T.

Also provided is a primer having the sequence set forth in SEQ ID NO: 19.

Further provided are nucleic acid vectors comprising nucleic acid molecules as described above and cells containing these vectors.

#### 25 **AKAP10-7 nucleic acids**

The nucleotide sequence of chromosome 17 containing the genomic sequence of the predominant allele (i.e., the allele present in the greatest frequency for a given population) of the AKAP-10 gene is presented as SEQ ID NO: 17. The nucleotide sequence of chromosome 17 containing the genomic  
30 sequence of the allelic variant AKAP10-7 is presented as SEQ ID NO: 14.

Provided herein is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as nucleotides of position 83,580 to position

156,577 of SEQ ID NO: 17, except that the nucleotide at position 129600 of SEQ ID NO: 17 is replaced with a nucleotide selected from among A, C and T.

Further provided is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as nucleotides of position 83,580 to position  
5 156,577 of SEQ ID NO: 17, except that the nucleotide at position 129600 of SEQ ID NO: 17 is replaced with the nucleotide A.

Also provided is an isolated nucleic acid molecule comprising the sequence of nucleotides set forth as nucleotides of position 83,580 to position 156,577 of SEQ ID NO: 14.

10 Further provided is an isolated nucleic acid molecule, comprising at least 14 or 16 contiguous nucleotides of SEQ. ID. NO 14; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides from position 129556 to position 129604 of SEQ. ID. NO: 14.

Also provided is an isolated nucleic acid molecule, comprising at least 30  
15 contiguous nucleotides of SEQ. ID. NO 14; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides from position 129556 to position 29604 of SEQ. ID. NO: 14.

Also provided is an isolated nucleic acid molecule, comprising at least 50 contiguous nucleotides of SEQ. ID. NO 14; wherein the contiguous nucleotides  
20 include a sequence of 5 contiguous nucleotides from position 129556 to position 129604 of SEQ. ID. NO: 14.

Further provided are primers, probes or antisense nucleic acid molecules that specifically hybridize adjacent to or at a polymorphic region spanning a position corresponding to position 129,600 of SEQ ID NO 14 or 17 of an  
25 AKAP10 allele or the complement thereof.

Further provided are primers that specifically hybridizes at a position immediately adjacent to a position corresponding to position 129600 of SEQ ID NO: 14 or 17 of an AKAP10 allele.

Also provided are primers that are extended by a nucleotide that  
30 specifically base pairs with the nucleotide at a position corresponding to position 129600 of SEQ ID NO: 14 of an AKAP10 allele.

Also provided are such primers, probes or antisense nucleic acid molecules as described above that hybridize under moderate or high conditions.

Further provided are such primers, probes or antisense nucleic acid molecules as described above, that are single-stranded and comprise at least 14  
5 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5 contiguous nucleotides from nucleotide position 129556 to position 129604 of SEQ. ID. NO: 14. The primers, probes or antisense nucleic acid molecules can also comprise at least 20 or 30 contiguous nucleotides of SEQ ID NO: 14, or the complement thereof.

10 Further provided are such primers, probes or antisense nucleic acid molecules as described above, that are single-stranded and comprise at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5 contiguous nucleotides from nucleotide position 129556 to position 129604 of SEQ. ID. NO: 17, except  
15 that the nucleotide at position 129600 of SEQ ID NO: 17 is replaced with a nucleotide selected from among A, C and T.

Also provided is a primer consisting essentially of the nucleic acid of SEQ ID NO: 20.

Further provided are nucleic acid vectors comprising nucleic acid  
20 molecules as described above and cells containing these vectors.

#### **Methods for detection of AKAP10 allelic variants**

Also provided are methods for determining the presence or absence of an allelic variant of a human AKAP10 gene, comprising determining the identity of the nucleotide at a position corresponding to position 2073 (of SEQ ID NO: 1) of  
25 the coding sequence of a human AKAP10 gene or the complement thereof, wherein the variant has a nucleotide other than A at a position corresponding to position 2073 (of SEQ ID NO 1).

Further provided is a method comprising hybridizing a target nucleic acid comprising a human AKAP10-encoding nucleic acid or fragment thereof or a  
30 complement of a human AKAP10-encoding nucleic acid or fragment thereof with a nucleic acid primer that hybridizes adjacent to a position corresponding to position 2073 (of SEQ ID NO: 1) of the coding sequence of the human AKAP10

gene or the complement thereof; extending the nucleic acid primer using the target nucleic acid as a template; and determining the mass of the extended primer to identify the nucleotide present at a position corresponding to position 2073 or the complement thereof, thereby determining the presence or absence  
5 of the allelic variant. The mass of the extended primer can be determined by mass spectrometry.

Further provided is a method that includes the steps of hybridizing a target nucleic acid comprising a human AKAP10-encoding nucleic acid or fragment thereof with a nucleic acid primer that hybridizes adjacent to a position  
10 corresponding to position 2073 (of SEQ ID NO: 1) of the coding sequence of the human AKAP10 gene or the complement thereof; extending the nucleic acid primer using the target nucleic acid as a template in the presence of at least one dideoxynucleotide; and determining the mass of the extended primer to identify the nucleotide present at a position corresponding to position 2073 or the  
15 complement thereof, thereby determining the presence or absence of an allelic variant. The dideoxynucleotide can be, but are not limited to, ddT or ddA. The primer can also be extended in the presence at least two dideoxynucleotides which are ddT and ddC or ddA and ddG. The mass of the extended primer can be determined by mass spectrometry. Also provided are the above described  
20 methods wherein hybridization is effected under conditions of high stringency.

Further provided is a method for detecting the presence or absence of an allelic variant of a human AKAP10 gene, comprising determining the identity of the nucleotide at a position corresponding to position 2073 (of SEQ ID NO: 1) of the coding sequence of a human AKAP10 gene or the complement thereof,  
25 wherein a variant has a nucleotide other than A at a position corresponding to position 2073 comprising hybridizing a target nucleic acid comprising a human AKAP10-encoding nucleic acid, complement thereof or fragment thereof with a single-stranded nucleic acid probe at a position corresponding to position 2073 of the coding sequence of the human AKAP10 gene or complement thereof; and  
30 detecting hybridized probe to identify the nucleotide present at a position corresponding to position 2073 or the complement thereof, thereby determining the presence or absence of an allelic variant.

Further provided is a method as described above wherein hybridization is effected under conditions of high stringency.

Also provided is a method as described above, wherein the nucleotide of the probe that hybridizes with the nucleotide at a position corresponding to  
5 position 2073 is complementary to a G, T, or C nucleotide or the nucleotide of the probe that hybridizes with the nucleotide at the complement of a position corresponding to position 2073 is complementary to a G, A, or C nucleotide.

Also provided are methods where the nucleotide detected at a position corresponding to position 2073 is a G and where the nucleotide detected at the  
10 complement of a position corresponding to position 2073 is a C.

Further provided are methods as described above to determine the presence or absence of other allelic variants of the human AKAP10 gene at *polymorphic regions corresponding to position 83587 (a nucleotide other than C), corresponding to position 129600 (a nucleotide other than G) and*  
15 *corresponding to position 156,277 (a nucleotide other than T) of SEQ ID NO: 17.*

**Methods for determining susceptibility to morbidity and/or increased or early mortality**

Also provided are methods for determining susceptibility to morbidity,  
20 increased or early mortality, or morbidity and increased or early mortality of a subject. These methods include the steps of detecting the presence or absence of at least one allelic variant of a polymorphic region of an AKAP10 gene that is associated with susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality; wherein the predominant allele  
25 includes an A at a position corresponding to position 2073 of SEQ ID NO: 1, and the presence of the allelic variant is indicative of increased susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality as compared to the susceptibility of a subject who does not comprise the allelic variant. The polymorphic region of the AKAP10 gene can comprise a  
30 nucleotide other than an A at a position corresponding to position 2073 of the coding sequence of the AKAP10 gene or other than a T of the complement of the coding sequence of the AKAP10 gene.

Other polymorphic regions of the AKAP10 gene representing allelic variants of the AKAP10 gene that are associated with susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality comprise a nucleotide other than a C at a position corresponding to position

5 83587 of the SEQ ID NO: 17 or other than a G on the complementary strand, a nucleotide other than a G at a position corresponding to position 129600 of the SEQ ID NO: 17 or other than a C on the complementary strand and a nucleotide other than T at a position corresponding to position 156,277 of SEQ ID NO: 17 or other than A on the complementary strand.

10 The detection of the presence or absence of an allelic variant can be effected by, but is not limited to, methods such as allele specific hybridization, primer specific extension, oligonucleotide ligation assay, restriction enzyme site analysis and single-stranded conformation polymorphism analysis. The detection can further be effected, but is not limited to, by mass spectrometry or by a  
15 signal moiety such as radioisotopes, enzymes, antigens, antibodies, spectrophotometric reagents, chemiluminescent reagents, fluorescent reagents and other light producing reagents.

A collection of polymorphic regions of the AKAP10 gene that individually represent allelic variants associated with morbidity, increased or early mortality,  
20 or morbidity and increased or early mortality in a subject may be more informative than a single allelic variant. Each allelic variant may be assayed individually or the collection may be assayed simultaneously using multiplex assay methods. Further provided is a collection of polymorphic regions of the AKAP10 gene comprising at least two polymorphic regions selected from among  
25 a position corresponding to position 2073 of SEQ ID NO 3, a position corresponding to position 83587 of SEQ ID NO: 13, a position corresponding to position 129,600 of SEQ ID NO: 14 and a position corresponding to position 156,277 of SEQ ID NO: 18.

#### Cells

30 Further provided is a cell comprising a heterologous nucleic acid, that encodes a human AKAP-10 variant protein or portion thereof that exhibits a biological activity of the full length variant protein, wherein the AKAP-10 protein

or portion thereof has a valine at a position corresponding to amino acid residue position 646 of SEQ ID NO 2.

Also provided is a cell contains a heterologous nucleic acid, that encodes the amino acid sequence set forth in SEQ. ID. NO: 4.

- 5 Further provided is a cell comprising a heterologous nucleic acid, that includes the sequence of nucleotides as set forth from position 138 to position 2126 of SEQ. ID. NO: 3.

- Also provided is a cell comprising a heterologous nucleic acid, that includes the sequence of nucleotides as set forth from position 83580 to  
10 position 156,577 of SEQ. ID. NO: 13.

Further provided is a cell comprising a heterologous nucleic acid, that includes the sequence of nucleotides as set forth from position 83580 to position 156,577 of SEQ. ID. NO: 14.

- Also provided is a cell comprising a heterologous nucleic acid, that  
15 includes the sequence of nucleotides as set forth from position 83580 to position 156,577 of SEQ ID NO: 18.

#### Kits

- Further provided are kits for determining whether a subject has an increased susceptibility to morbidity and/or a predisposition for premature or  
20 increased or early mortality. The kits include a first primer or probe that specifically hybridizes adjacent to or at a polymorphic region spanning a position corresponding to position 2073 of SEQ ID NO 1 or 3 of an AKAP10 allele or the complement thereof and a second primer or probe that specifically hybridizes adjacent to or at a polymorphic region spanning a position corresponding to  
25 positions selected from among position 83587 of SEQ ID NO 13 or 17, position 129600 of SEQ ID NO 14 or 17, and position 156,277 of SEQ ID NO 18 or 17 of an AKAP10 allele or the complement thereof. Primers include, but are not limited to, nucleic acids having the sequence of nucleotides set forth in any of SEQ ID NO: 8, SEQ ID NO: 15, SEQ ID NO: 19 and SEQ ID NO 20. The kits  
30 also optionally contain instructions for carrying out assays, interpreting results or for aid in determining if a subject has a predisposition for an increased susceptibility to morbidity and/or a predisposition for premature or increased or

early mortality. The kits can also include at least one didieoxynucleotide such as ddA, ddC, ddG.

#### **Proteins and methods of producing**

Further provided is a method of producing a protein by growing a cell  
5 comprising a nucleic acid vector comprising a nucleic acid molecule, comprising a sequence of nucleotides that encodes the polypeptide of SEQ ID NO: 2, except that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with Val, Leu or Phe, under conditions whereby the protein is expressed and isolating the protein.

Also provided are methods wherein the cell is a mammalian cell, yeast  
10 cell, insect cell, bacterial cell or human cell.

Proteins produced by the above described methods are also provided.

#### **Transgenic animals**

Also provided are transgenic animals comprising heterologous nucleic acid encoding a human AKAP10 protein or portion thereof comprising the binding  
15 region for protein kinase A, wherein the AKAP10 protein or portion thereof includes valine at a position corresponding to position 646 of SEQ ID NO: 2, wherein the transgenic nucleotide acid is expressed; and, as a result of the expression, the transgenic animal has an alteration in cellular signal transduction. The transgenic animals include, but are not limited to, rodents, including rats and  
20 mice.

Further provided are transgenic animals comprising heterologous nucleic acid encoding the sequence of an allelic variant of a human AKAP10 gene comprising a nucleotide other than a C at a position corresponding with position 83587 of the SEQ ID NO: 17, a nucleotide other than a G at a position  
25 corresponding to position 129600 of the SEQ ID NO: 17 or a nucleotide other than T at position corresponding to position 156,277 of SEQ ID NO: 17.

#### **Methods for identifying molecules that modulate the activity of AKAP10 variant proteins and nucleic acids**

Further provided are methods for identifying a molecule that modulates  
30 the biological activity of an AKAP10 protein. The method is practiced by combining the candidate molecule with a cell containing a nucleic acid molecule encoding an AKAP10 variant protein or portion thereof that retains a biological



- activity of the full length variant protein and that has an amino acid residue at a position corresponding to position 646 of SEQ ID NO: 2 which is not Ile, operably linked to a promoter such that the nucleotide sequence is expressed as an AKAP protein or portion thereof in the cell; and determining the affect of the
- 5 molecule upon the biological activity of the AKAP10 protein or portion thereof.

Also provided are methods as described above in which the activity of the AKAP10 protein or portion thereof is determined by examining signal transduction in the cell or by examining binding of AKAP10 protein or portion thereof to protein kinase A or by examining cellular phosphorylation.

- 10 Nucleic acid encoding other allelic variants of a human AKAP10 gene, that are not in coding regions, can be introduced into cells allowing for the identification of molecules that may modulate the expression of these sequences at the level of transcription, translation or processing. Such sequences of other allelic variant of a human AKAP10 gene can comprise a nucleotide other than a
- 15 C at a position corresponding with position 83587 of the SEQ ID NO: 17, a nucleotide other than a G at a position corresponding to position 129600 of the SEQ ID NO: 17 or a nucleotide other than T at position corresponding to position 156,277 of SEQ ID NO: 17. Assays to monitor transcription, translation and processing are familiar to produce in the field.

20 **Methods for indicating and/or predicting a susceptibility to an alteration in signal transduction**

- Further provided are methods for indicating an alteration in signal transduction and/or predicting a susceptibility to an alteration in signal transduction in a subject comprising detecting the presence or absence of an
- 25 allelic variant of an AKAP10 gene having a nucleotide other than A at a position corresponding to position 2073 of SEQ ID NO: 1, wherein the presence of a nucleotide other than A is indicative of an alteration in signal transduction.

Also provided are methods as described above wherein the allelic variant has a G at a position corresponding to position 2073 of SEQ ID NO: 1.

- 30 Other polymorphic regions of the AKAP10 gene representing allelic variants of the AKAP10 gene that are associated with an alteration in signal transduction comprise a nucleotide other than a C at a position corresponding

with position 83587 of the SEQ ID NO: 17 or other than a G on the complementary strand, a nucleotide other than a G at a position corresponding to position 129600 of the SEQ ID NO: 17 or other than a C on the complementary strand and a nucleotide other than T at position corresponding to position 156,277 of SEQ ID NO: 17 or other than A on the complementary strand.

Further provided are methods which also comprise detecting the presence or absence of an allelic variant at another polymorphic position of the AKAP10 gene selected from among a position corresponding to position 83587 of SEQ ID NO: 13, a position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18.

The alteration in signal transduction maybe related to disorders such as cardiovascular disorders, cardiac disorders, proliferative disorders, neurological disorders, neurodegenerative disorders, obesity, diabetes and peripheral retinopathies, Alzheimer's disease, altered left ventricular function, cardiomyopathies, bipolar disorder and retinitis pigmentosa.

Further provided are the above described methods for which the detecting step is by allele specific hybridization, primer specific extension, oligonucleotide ligation assay, restriction enzyme site analysis and single-stranded conformation polymorphism analysis.

Also provided are methods wherein the detecting is effected by mass spectrometry.

Further provided are methods wherein the detecting is effected by a signal moiety such as radioisotopes, enzymes, antigens, antibodies, spectrophotometric reagents, chemiluminescent reagents, fluorescent reagents and other light producing reagents.

#### **Supports**

Also provided are solid supports the include a nucleic acid containing a polymorphic region of an AKAP10 gene. The polymorphic region includes a nucleotide at a position corresponding to position 2073 of SEQ ID NO: 1 that is other than an A or other than T on the complementary strand.

Further provided is a solid support comprising a nucleic acid comprising a polymorphic region of an AKAP10 gene, wherein the polymorphic regions includes a nucleotide at a position corresponding to position 83587 of SEQ ID NO: 17 that is other than a C or other than G on the complementary strand.

- 5        Also provided is a solid support comprising a nucleic acid comprising a polymorphic region of an AKAP10 gene, wherein the polymorphic regions includes a nucleotide at a position corresponding to position 129600 of SEQ ID NO 17 that is other than a G or other than C on the complementary strand.

- 10       Also provided is a solid support comprising a nucleic acid comprising a polymorphic region of an AKAP10 gene, wherein the polymorphic regions includes a nucleotide at a position corresponding to position 156,277 of SEQ ID NO: 17 that is other than T or other than A on the complementary strand.

Further provided is a solid support comprising a nucleic acid comprising a polymorphic region of an AKAP10 gene which is a microarray.

- 15       Also provided is a microarray comprising at least 2 nucleic acid molecules each comprising a sequence of a polymorphic region of an AKAP10 gene at a position corresponding the position of the group consisting position 2073 of SEQ ID NO: 3, position 83587 of SEQ ID NO: 13, position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18., or the complements thereof.

- 20       Micrarrays are well known (see, *e.g.*, U.S. Patent Nos. 5,837,832; 5,858,659; 6,043,136; 6,043,031 and 6,156,501).

#### **Ribozymes**

- 25       Also provided is an anti-AKAP10 ribozyme comprising a sequence complementary to a polymorphic region of an AKAP10 gene. The polymorphic regions are selected from among a position corresponding to position 2073 of SEQ ID NO: 3, position 83587 of SEQ ID NO: 13, position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18.

Further provided is a ribozyme comprising the nucleic acid of SEQ ID NO: 25.

## DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

### A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents, patent applications, published applications and publications referred to throughout the disclosure herein are, unless noted otherwise, incorporated by reference in their entirety. In the event that there are a plurality of definitions for terms herein, those in this section prevail.

As used herein, sequencing refers to the process of determining a nucleotide sequence and can be performed using any method known to those of skill in the art. For example, if a polymorphism is identified or known, and it is desired to assess its frequency or presence in nucleic acid samples taken from the subjects that comprise the database, the region of interest from the samples can be isolated, such as by PCR or restriction fragments, hybridization or other suitable method known to those of skill in the art, and sequenced. For purposes herein, sequencing analysis is preferably effected using mass spectrometry (see, *e.g.*, U.S. Patent Nos. 5,547,835, 5,622,824, 5,851,765, and 5,928,906). Nucleic acids can also be sequenced by hybridization (see, *e.g.*, U.S. Patent Nos. 5,503,980, 5,631,134, 5,795,714) and including analysis by mass spectrometry (see, U.S. Application Serial Nos. 08/419,994 and 09/395,409). Alternatively, sequencing may be performed using other known methods, such as set forth in U.S. Patent Nos. 5,525,464; 5,695,940; 5,834,189; 5,869,242; 5,876,934; 5,908,755; 5,912,118; 5,952,174; 5,976,802; 5,981,186; 5,998,143; 6,004,744; 6,017,702; 6,018,041; 6,025,136; 6,046,005; 6,087,095; 6,117,634, 6,013,431, WO 98/30883; WO 98/56954; WO 99/09218; WO/00/58519, and the others.

As used herein, "polymorphism" refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, *i.e.*, two different nucleotide sequences, is referred to as a "polymorphic region of a gene". A polymorphic region can be a single

nucleotide, the identity of which differs in different alleles. A polymorphic region can also be several nucleotides in length.

As used herein, "polymorphic gene" refers to a gene having at least one polymorphic region.

- 5       As used herein, "allele", which is used interchangeably herein with "allelic variant" refers to alternative forms of a gene or portions thereof. Alleles occupy the same locus or position on homologous chromosomes. When a subject has two identical alleles of a gene, the subject is said to be homozygous for the gene or allele. When a subject has two different alleles of a gene, the subject is said
- 10   to be heterozygous for the gene. Alleles of a specific gene can differ from each other in a single nucleotide, or several nucleotides, and can include substitutions, deletions, and insertions of nucleotides. An allele of a gene can also be a form of a gene containing a mutation.

- As used herein, "predominant allele" refers to an allele that is represented
- 15   in the greatest frequency for a given population. The allele or alleles that are present in lesser frequency are referred to as allelic variants.

- As used herein, "associated" refers to coincidence with the development or manifestation of a disease, condition or phenotype. Association may be due to, but is not limited to, genes responsible for housekeeping functions whose
- 20   alteration can provide the foundation for a variety of diseases and conditions, those that are part of a pathway that is involved in a specific disease, condition or phenotype and those that indirectly contribute to the manifestation of a disease, condition or phenotype.

- As used herein, the term "subject" refers to mammals and in particular
- 25   human beings.

- As used herein, the term "gene" or "recombinant gene" refers to a nucleic acid molecule comprising an open reading frame and including at least one exon and (optionally) an intron sequence. A gene can be either RNA or DNA. Genes may include regions preceding and following the coding region
- 30   (leader and trailer).

      As used herein, "intron" refers to a DNA sequence present in a given gene which is spliced out during mRNA maturation.

As used herein, "nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: x" refers to the nucleotide sequence of the complementary strand of a nucleic acid strand having SEQ ID NO: x. The term "complementary strand" is used herein interchangeably with the term

5 "complement". The complement of a nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand. When referring to double stranded nucleic acids, the complement of a nucleic acid having SEQ ID NO: x refers to the complementary strand of the strand having SEQ ID NO: x or to any nucleic acid having the nucleotide sequence of the complementary strand

10 of SEQ ID NO: x. When referring to a single stranded nucleic acid having the nucleotide sequence SEQ ID NO: x, the complement of this nucleic acid is a nucleic acid having a nucleotide sequence which is complementary to that of SEQ ID NO: x.

As used herein, the term "coding sequence" refers to that portion of a

15 gene that encodes an amino acid sequence of a protein.

As used herein, the term "sense strand" refers to that strand of a double-stranded nucleic acid molecule that has the sequence of the mRNA that encodes the amino acid sequence encoded by the double-stranded nucleic acid molecule.

As used herein, the term "antisense strand" refers to that strand of a

20 double-stranded nucleic acid molecule that is the complement of the sequence of the mRNA that encodes the amino acid sequence encoded by the double-stranded nucleic acid molecule.

As used herein, the amino acids, which occur in the various amino acid sequences appearing herein, are identified according to their well-known, three-

25 letter or one-letter abbreviations. The nucleotides, which occur in the various DNA fragments, are designated with the standard single-letter designations used routinely in the art (see, Table 1).

As used herein, amino acid residue refers to an amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The

30 amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the

polypeptide. NH<sub>2</sub> refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxyl terminus of a polypeptide. In keeping with standard polypeptide nomenclature described in *J. Biol. Chem.*, 243:3552-59 (1969) and adopted at 37 C.F.R. § 5

1.821 - 1.822, abbreviations for amino acid residues are shown in the following Table:

**Table 1**  
Table of Correspondence

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine

SYMBOL		
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

- 5 It should be noted that all amino acid residue sequences represented herein by formulae have a left to right orientation in the conventional direction of amino-terminus to carboxyl-terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those referred
- 10 to in 37 C.F.R. § § 1.821-1.822, and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or to an amino-terminal group such as NH<sub>2</sub> or to a carboxyl-terminal group such as COOH.
- 15 In a peptide or protein, suitable conservative substitutions of amino acids are known to those of skill in this art and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (see, e.g.,
- 20 Watson et al. *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. co., p.224).

Such substitutions are preferably made in accordance with those set forth in TABLE 2 as follows:



TABLE 2

	Original residue	Conservative substitution
	Ala (A)	Gly; Ser
	Arg (R)	Lys
5	Asn (N)	Gln; His
	Cys (C)	Ser
	Gln (Q)	Asn
	Glu (E)	Asp
	Gly (G)	Ala; Pro
10	His (H)	Asn; Gln
	Ile (I)	Leu; Val
	Leu (L)	Ile; Val
	Lys (K)	Arg; Gln; Glu
	Met (M)	Leu; Tyr; Ile
15	Phe (F)	Met; Leu; Tyr
	Ser (S)	Thr
	Thr (T)	Ser
	Trp (W)	Tyr
	Tyr (Y)	Trp; Phe
20	Val (V)	Ile; Leu

Other substitutions are also permissible and may be determined empirically or in accord with known conservative substitutions.

As used herein, a DNA or nucleic acid homolog refers to a nucleic acid that includes a preselected conserved nucleotide sequence, such as a sequence encoding a therapeutic polypeptide. By the term "substantially homologous" is meant having at least 80%, preferably at least 90%, most preferably at least 95% homology therewith or a less percentage of homology or identity and conserved biological activity or function.

The terms "homology" and "identity" are often used interchangeably. In this regard, percent homology or identity may be determined, for example, by comparing sequence information using a GAP computer program. The GAP program uses the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443 (1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482 (1981)). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program may include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted

comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745 (1986), as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

Whether any two nucleic acid molecules have nucleotide sequences that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% "identical" can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444 (1988). Alternatively the BLAST function of the National Center for Biotechnology Information database may be used to determine identity

In general, sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. (See, e.g.: *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H. & Lipton, D., *SIAM J Applied Math* 48:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to *Human Genome Computing*, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H. & Lipton, D., *SIAM J Applied Math* 48:1073 (1988). Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J.,

*et al.*, *Nucleic Acids Research* 12(II):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., *et al.*, *J Molec Biol* 215:403 (1990)).

Therefore, as used herein, the term "identity" represents a comparison between a test and a reference polypeptide or polynucleotide. For example, a  
5 test polypeptide may be defined as any polypeptide that is 90% or more identical to a reference polypeptide.

As used herein, the term at least "90% identical to" refers to percent identities from 90 to 99.99 relative to the reference polypeptides. Identity at a level of 90% or more is indicative of the fact that, assuming for exemplification  
10 purposes a test and reference polynucleotide length of 100 amino acids are compared. No more than 10% (i.e., 10 out of 100) amino acids in the test polypeptide differs from that of the reference polypeptides. Similar comparisons may be made between a test and reference polynucleotides. Such differences may be represented as point mutations randomly distributed over the entire  
15 length of an amino acid sequence or they may be clustered in one or more locations of varying length up to the maximum allowable, e.g. 10/100 amino acid difference (approximately 90% identity). Differences are defined as nucleic acid or amino acid substitutions, or deletions.

As used herein, stringency conditions refer to the washing conditions for  
20 removing the non-specific probes and conditions that are equivalent to either high, medium, or low stringency as described below:

- 1) high stringency: 0.1 x SSPE, 0.1% SDS, 65°C
- 2) medium stringency: 0.2 x SSPE, 0.1% SDS, 50°C
- 3) low stringency: 1.0 x SSPE, 0.1% SDS, 50°C.

25 It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures. Specific hybridization is generally effected at medium or high stringency and refers to preferential hybridization to a targeted sequence compared to hybridization to a non-targeted sequence, such that the presence of the targeted sequence can be detected.

30 As used herein, "heterologous DNA" is DNA that encodes RNA and proteins that are not normally produced *in vivo* by the cell in which it is expressed or that mediates or encodes mediators that alter expression of

endogenous DNA by affecting transcription, translation, or other regulatable biochemical processes or is not present in the exact orientation or position as the counterpart DNA in a wildtype cell. Heterologous DNA may also be referred to as foreign DNA. Any DNA that one of skill in the art would recognize or  
5 consider as heterologous or foreign to the cell in which is expressed is herein encompassed by heterologous DNA. Examples of heterologous DNA include, but are not limited to, DNA that encodes traceable marker proteins, such as a protein that confers drug resistance, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that  
10 encodes other types of proteins, such as antibodies. Antibodies that are encoded by heterologous DNA may be secreted or expressed on the surface of the cell in which the heterologous DNA has been introduced.

As used herein, isolated with reference to a nucleic acid molecule or polypeptide or other biomolecule means that the nucleic acid or polypeptide has  
15 separated from the genetic environment from which the polypeptide or nucleic acid were obtained. It may also mean altered from the natural state. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed  
20 herein. Thus, a polypeptide or polynucleotide produced and/or contained within a recombinant host cell is considered isolated. Also intended as an "isolated polypeptide" or an "isolated polynucleotide" are polypeptides or polynucleotides that have been purified, partially or substantially, from a recombinant host cell or from a native source. For example, a recombinantly produced version of a  
25 compounds can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). The terms isolated and purified are sometimes used interchangeably.

Thus, by "isolated" is meant that the nucleic acid molecule is free of the coding sequences of those genes that, in the naturally-occurring genome of the  
30 organism (if any) immediately flank the gene encoding the nucleic acid of interest. Isolated DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, recombinant hybrid DNA, or synthetic DNA. It may be

identical to a native DNA sequence, or may differ from such sequence by the deletion, addition, or substitution of one or more nucleotides.

Isolated or purified as it refers to preparations made from biological cells or hosts means any cell extract containing the indicated DNA or protein including  
5 a crude extract of the DNA or protein of interest. For example, in the case of a protein, a purified preparation can be obtained following an individual technique or a series of preparative or biochemical techniques and the DNA or protein of interest can be present at various degrees of purity in these preparations. The procedures may include for example, but are not limited to, ammonium sulfate  
10 fractionation, gel filtration, ion exchange change chromatography, affinity chromatography, density gradient centrifugation and electrophoresis.

A preparation of DNA or protein that is "substantially pure" or "isolated" should be understood to mean a preparation free from naturally occurring materials with which such DNA or protein is normally associated in nature.  
15 "Essentially pure" should be understood to mean a "highly" purified preparation that contains at least 95% of the DNA or protein of interest.

A cell extract that contains the DNA or protein of interest should be understood to mean a homogenate preparation or cell-free preparation obtained from cells that express the protein or contain the DNA of interest. The term  
20 "cell extract" is intended to include culture media, especially spent culture media from which the cells have been removed.

As used herein, receptor refers to a biologically active molecule that specifically binds to (or with) other molecules. The term "receptor protein" may be used to more specifically indicate the proteinaceous nature of a specific  
25 receptor.

As used herein, recombinant refers to any progeny formed as the result of genetic engineering.

As used herein, a promoter region refers to the portion of DNA of a gene that controls transcription of the DNA to which it is operatively linked. The  
30 promoter region includes specific sequences of DNA that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region

includes sequences that modulate this recognition, binding and transcription initiation activity of the RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated.

- 5           As used herein, the phrase "operatively linked" generally means the sequences or segments have been covalently joined into one piece of DNA, whether in single or double stranded form, whereby control or regulatory sequences on one segment control or permit expression or replication or other such control of other segments. The two segments are not necessarily
- 10   contiguous. For gene expression a DNA sequence and a regulatory sequence(s) are connected in such a way to control or permit gene expression when the appropriate molecular, e.g., transcriptional activator proteins, are bound to the regulatory sequence(s).

- As used herein, production by recombinant means by using recombinant
- 15   DNA methods means the use of the well known methods of molecular biology for expressing proteins encoded by cloned DNA, including cloning expression of genes and methods, such as gene shuffling and phage display with screening for desired specificities.

- As used herein, the term "conjugated" refers stable attachment, such as
- 20   ionic or covalent attachment.

          As used herein, a composition refers to any mixture of two or more products or compounds. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

- As used herein, a combination refers to any association between two or
- 25   more items.

          As used herein, substantially identical to a product means sufficiently similar so that the property of interest is sufficiently unchanged so that the substantially identical product can be used in place of the product.

- As used herein, the term "vector" refers to a nucleic acid molecule
- 30   capable of transporting another nucleic acid to which it has been linked. One type of preferred vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Preferred vectors are those capable of

autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the

5 form of "plasmids" which refer generally to circular double stranded DNA loops which, in their vector form are not bound to the chromosome. "Plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. Other such other forms of expression vectors that serve equivalent functions and that become known in the art subsequently hereto.

10 As used herein, "indicating" or "determining" means that the presence or absence of an allelic variant may be one of many factors that are considered when a subject's predisposition to a disease or disorder is evaluated. Thus a predisposition to a disease or disorder is not necessarily conclusively determined by only ascertaining the presence or absence of one or more allelic variants, but

15 the presence of one of more of such variants is among a number of factors considered.

As used herein, "predisposition to develop a disease or disorder" means that a subject having a particular genotype and/or haplotype has a higher likelihood than one not having such a genotype and/or haplotype for developing

20 a particular disease or disorder.

As used herein, "morbidity" refers to conditions, such as diseases or disorders, that compromise the health and well-being of an organism, such as an animal. Morbidity susceptibility or morbidity-associated genes are genes that, when altered, for example, by a variation in nucleotide sequence, facilitate the

25 expression of a specific disease clinical phenotype. Thus, morbidity susceptibility genes have the potential, upon alteration, of increasing the likelihood or general risk that an organism will develop a specific disease.

As used herein, "mortality" refers to the statistical likelihood that an organism, particularly an animal, will not survive a full predicted lifespan.

30 Hence, a trait or a marker, such as a polymorphism, associated with increased mortality is observed at a lower frequency in older than younger segments of a population.

As used herein, "transgenic animal" refers to any animal, preferably a non-human animal, *e.g.* a mammal, bird or an amphibian, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art.

- 5 The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This
- 10 molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animals described herein, the transgene causes cells to express a recombinant form of a protein. However, transgenic animals in which the recombinant gene is silent are also contemplated, as for example, using the FLP or CRE recombinase dependent
- 15 constructs. Moreover, "transgenic animal" also includes those recombinant animals in which gene disruption of one or more genes is caused by human intervention, including recombination and antisense techniques.

As used herein, "target nucleic acid" refers to a nucleic acid molecule which contains all or a portion of a polymorphic region of a gene of interest.

- 20 As used herein, "signal moiety" refers to any moiety that allows for the detection of a nucleic acid molecule. Included are moieties covalently attached to nucleic acids and those that are not.

- As used herein, "molecule that modulates or effects the biological activity of an AKAP10 protein" refers to any drug, small molecule, nucleic acid (sense and antisense), ribozyme, protein, peptide, lipid, carbohydrate *etc.* or
- 25 combination thereof, that directly or indirectly changes, alters, abolishes, increases or decreases a biological activity attributed to AKAP10 protein.

- As used herein, "biological activity of an AKAP10 protein" refers to, but is not limited to, binding of AKAP10 to protein kinase A or its subunits,
- 30 localization of AKAP10 protein to a subcellular site, *e.g.*, the mitochondria, localization of protein kinase A to the mitochondria and binding of AKAP10 protein to other proteins including other signalling enzymes.



As used herein, "combining" refers to contacting the biologically active agent with a cell or animal such that the agent is introduced into the cell or animal. For a cell any method that results in an agent traversing the plasma membrane is useful. For an animal any of the standard routes of administration of an agent, *e.g.* oral, rectal, transmucosal, intestinal, intravenous, intraperitoneal, intraventricular, subcutaneous, intramuscular, *etc.*, can be used.

As used herein, a composition refers to any mixture. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

As used herein, a combination refers to any association between two or among more items.

As used herein, "kit" refers to a package that contains a combination, such as one or more primers or probes used to amplify or detect polymorphic regions of AKAP10 genes, optionally including instructions and/or reagents for their use.

As used herein, "solid support" refers to a support substrate or matrix, such as silica, polymeric materials or glass. At least one surface of the support can be partially planar. Regions of the support may be physically separated, for example with trenches, grooves, well or the like. Some examples of solid supports include slides and beads. Supports are of such composition so as to allow for the immobilization or attachment of nucleic acids and other molecules such that these molecules retain their binding ability.

As used herein, "array" refers to a collection of elements, such as nucleic acids, containing three or more members. An addressable array is one in which the members of the array are identifiable, typically by position on a solid support. Hence, in general the members of the array will be immobilized to discrete identifiable loci on the surface of a solid phase.

As used herein, "specifically hybridizes" refers to hybridization of a probe or primer only to a target sequence preferentially to a non-target sequence. Those of skill in the art are familiar with parameters that affect hybridization; such as temperature, probe or primer length and composition, buffer composition

and salt concentration and can readily adjust these parameters to achieve specific hybridization of a nucleic acid to a target sequence.

As used herein "nucleic acid" refers to polynucleotides such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The term should also  
5 be understood to include, as equivalents, derivatives, variants and analogs of either RNA or DNA made from nucleotide analogs, single (sense or antisense) and double-stranded polynucleotides. Deoxyribonucleotides include deoxyadenosine, deoxycytidine, deoxyguanosine and deoxythymidine. For RNA, the uracil base is uridine.

10 As used herein, "mass spectrometry" encompasses any suitable mass spectrometric format known to those of skill in the art. Such formats include, but are not limited to, Matrix-Assisted Laser Desorption/Ionization, Time-of-Flight (MALDI-TOF), Electrospray (ES), IR-MALDI (see, *e.g.*, published International PCT Application No. WO 99/57318 and U.S. Patent No. 5,118,937) Ion  
15 Cyclotron Resonance (ICR), Fourier Transform and combinations thereof. MALDI, particular UV and IR, are among the preferred formats.

As used herein, "at a position corresponding to" refers to a position of interest (*i.e.*, base number or residue number) in a nucleic acid molecule or  
20 protein. Corresponding positions can be determined by comparing and aligning sequences to maximize the number of matching nucleotides or residues, for example, such that identity between the sequences is greater than 95%, preferably greater than 96%, more preferably greater than 97%, even more preferably greater than 98% and most preferably greater than 99%. The position  
25 of interest is then given the number assigned in the reference nucleic acid molecule. For example, it is shown herein that a particular polymorphism in AKAP-10 occurs at nucleotide 2073 of SEQ ID No. 1. To identify the corresponding nucleotide in another allele or isolate, the sequences are aligned and then the position that lines up with 2073 is identified. Since various alleles  
30 may be of different length, the position designate 2073 may not be nucleotide 2073, but instead is at a position that "corresponds" to the position in the reference sequence.

As used herein, "primer" and "probe" refer to a nucleic acid molecule including DNA, RNA and analogs thereof, including protein nucleic acids (PNA), and mixtures thereof. Such molecules are typically of a length such that they are statistically unique (*i.e.*, occur only once) in the genome of interest.

- 5 Generally, for a probe or primer to be unique in the human genome, it contains at least 14, 16 or contiguous nucleotides of a sequence complementary to or identical to a gene of interest. Probes and primers can be 10, 20, 30, 50, 100 or more nucleic acids long.

- As used herein, "antisense nucleic acid molecule" refers to a molecule  
10 encoding a sequence complementary to at least a portion of an RNA molecule. The sequence is sufficiently complementary to be able to hybridize with the RNA, preferably under moderate or high stringency conditions to form a stable duplex. The ability to hybridize depends on the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing  
15 nucleic acid, the more base mismatches with an RNA it can contain and still form a stable duplex. One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

- As used herein, a "variant protein" refers to a protein encoded by an  
20 allelic variant of a AKAP10 gene which results in a change of an amino acid residue at a particular position relative to that position in the protein encoded by the predominant allele.

- As used herein, "signal transduction" refers to the propagation of a signal. In general, an extracellular signal is transmitted through the cell  
25 membrane to become an intracellular signal. This signal can then stimulate a cellular response. The term also encompasses signals that are propagated entirely within a cell. The polypeptide molecules involved in signal transduction processes are typically receptor and non-receptor protein kinases, receptor and non-receptor protein phosphatases, nucleotide exchange factors and  
30 transcription factors. One of the key biochemical mechanisms involved in signal transduction is protein phosphorylation. AKAP10 proteins are involved in signal transduction. These proteins bind to protein kinase A (PKA) and anchor the

kinase at a location, such as at mitochondrial locations, where PKA acts to phosphorylate a specific substrate. Thus, an alteration in AKAP10 binding to PKA, localization to the mitochondria, or phosphorylation by PKA, among other steps will result in an alteration in signal transduction. Assays including those  
5 that determine phosphorylation by PKA, association of PKA and AKAP10 and localization of AKAP10 can be used to monitor the state of signal transduction.

As used herein, "adjacent" refers to a position 5' to the site of a single nucleotide polymorphism (SNP) such that there could be unpaired nucleotides between that position and the site of the SNP.

10 As used herein, "immediately adjacent" refers to a position 5' to the site of a single nucleotide polymorphism (SNP) such that there are no unpaired nucleotides between that position and the site of the SNP.

As used herein, "binding to PKA", refers to the interaction of the PKA binding domain of an AKAP10 protein and the regulatory subunits RI and/or RII  
15 of the protein kinase A holoenzyme.

#### **B. Polymorphic AKAPs**

Polymorphic sequences of A-kinase anchoring protein (AKAP) genes are provided herein. Also provided herein are polymorphic AKAP proteins encoded by polymorphic AKAP gene sequences. These polymorphic sequences are based  
20 in differences discovered in AKAP genes within and among different organisms, and, in particular, humans.

Polymorphisms of the genome can lead to altered gene function, protein function or mRNA instability. AKAPs provide a mechanism for regulating ubiquitous cAMP-dependent kinase (PKA) activity by tethering PKA to specific  
25 subcellular locations thereby segregating it with particular components in a given signaling pathway and contributing to specificity in cellular responses to extracellular signals. AKAPs thus play a fundamental role in the basic functioning of cells, the response of cells to their environment and ultimately in the coordination of vital systems within an organism. Therefore, polymorphisms  
30 in AKAP gene sequences may significantly affect the proper functioning of cells and systems within organisms and could be directly linked with certain disorders or could predispose an organism to a variety of diseases and disorders,

especially those involving alterations in cellular protein phosphorylation and/or signal transduction. Among such disorders and diseases are: neurodegenerative diseases, such as Alzheimer's Disease, cardiovascular disorders, cardiac disorders, particularly disorders associated with altered left ventricular function, cardiomyopathies, proliferative disorders, bipolar disorder and other neurological disorders, obesity, diabetes and certain peripheral retinopathies, such as retinitis pigmentosa. The discovery of AKAP gene polymorphisms, such as those described herein, provides for the identification and development of diagnostic and prognostic methods, also provided herein, and the development of drug therapies and treatment regimens. Furthermore, polymorphisms of AKAP genes aid in the study of AKAP protein structure and function, which also contributes to the development of diagnostic methods and therapies.

#### 1. AKAP10

The AKAP10 protein is primarily located in mitochondria. The sequence of a human AKAP10 cDNA (also referred to as D-AKAP2) is available in the GenBank database, at accession numbers AF037439 and NM 007202, and is provided in SEQ. ID. NO:1. The AKAP10 gene is located on chromosome 17.

The sequence of a mouse D-AKAP2 cDNA is also available in the GenBank database (see accession number AF021833). The mouse D-AKAP2 protein contains an RGS domain near the amino terminus that is characteristic of proteins that interact with G $\alpha$  subunits and possess GTPase activating protein-like activity (Huang *et al.* (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94:11184-11189). The human AKAP10 protein also has sequences homologous to RGS domains. The carboxy-terminal 40 residues of the mouse D-AKAP2 protein are responsible for the interaction with the regulatory subunits of PKA. This sequence is fairly well conserved between the mouse D-AKAP2 and human AKAP10 proteins.

## 2. Polymorphisms of the human AKAP10 gene and polymorphic AKAP10 proteins

Polymorphisms of AKAP genes that alter gene expression, regulation, protein structure and/or protein function are more likely to have a significant effect on the regulation of enzyme (particularly PKA) activity, cellular transduction of signals and responses thereto and on the basic functioning of cells than polymorphisms that do not alter gene and/or protein function. Included in the polymorphic AKAPs provided herein are human AKAP10 proteins containing differing amino acid residues at position number 646 of SEQ. ID. No.

10 2.

Amino acid 646 of the human AKAP10 protein (SEQ. ID. NO: 2) is located in the carboxy-terminal region of the protein within a segment that participates in the binding of R-subunits of PKAs. This segment includes the carboxy-terminal 40 amino acids.

15 The amino acid residue reported for position 646 of the human AKAP10 protein is an isoleucine. Polymorphic human AKAP10 proteins provided herein have the amino acid sequence set forth in SEQ. ID. NO: 2 but contain residues other than isoleucine at amino acid position 646 of the protein. In particular embodiments of the polymorphic human AKAP10 proteins provided herein, the amino acid at position 646 of SEQ. ID. NO: 2 is a valine (as set forth in SEQ. ID. NO: 4), leucine or phenylalanine residue.

### a. An A to G transition at nucleotide 2073 of the human AKAP10 coding sequence

As described herein, an allelic variant of the human AKAP10 gene is at the polymorphic site at position 2073 of the coding sequence (see SEQ.ID. NO: 3) and encodes a valine at position 646 of the AKAP10 protein. This allelic variant has been found to vary in frequency in DNA samples from younger and older segments of a healthy population. This allele has the A at position 2073 of the AKAP10 gene coding sequence of SEQ. ID. NO: 1 changed to a G, giving rise to the sequence set forth in SEQ. ID. NO: 3. Consequently, the codon for amino acid 646 changes from ATT, coding for isoleucine, to GTT, coding for valine.

**b. An A to C transversion at nucleotide 2073 of the human AKAP10 coding sequence**

In another human AKAP10 allelic variant, the nucleotide at position 2073 of the coding sequence in SEQ. ID. NO: 1 is changed from an A to a C. Thus, changing the codon for amino acid 646 from ATT, coding for isoleucine, to CTT, coding for leucine.

**c. An A to T transversion at nucleotide 2073 of the human AKAP10 coding sequence**

In another human AKAP10 allelic variant, the nucleotide at position 2073 of the coding sequence in SEQ. ID. NO: 1 is changed from an A to a T. Thus, the codon for amino acid 646 changes from ATT, coding for isoleucine, to TTT, coding for phenylalanine.

**d. Other AKAP10 polymorphisms**

TABLE 3

Name	GenBank Acession No.	SNP	Location
10-1	AC005730	T/C	156277
10-6	AC005730	C/G	83587
10-7	AC005730	G/A	129600

For AKAP10-1 additional variants are represented by the presence of A or G at nucleotide position 156277 of SEQ ID NO: 17.

For AKAP10-6 additional variants are represented by the presence of A or T at nucleotide position 83587 of SEQ ID NO: 17.

For AKAP10-7 additional variants are represented by the presence of C or T at nucleotide position 129600 of SEQ ID NO: 17.

**C. Isolation of Polymorphic AKAP10 Gene Sequences**

Exemplary nucleic acid sequences encoding polymorphic human AKAP10 proteins are represented by nucleotides which encode the amino acid sequence as set forth in SEQ. ID. NO: 3. Nucleic acid molecules encoding the sequence of amino acids set forth in SEQ. ID. NO: 1 in which amino acid 646 has been replaced with a leucine or phenylalanine are also provided herein.

Other exemplary nucleic acid sequences represent allelic variants of the AKAP10 gene which are not located in protein coding regions. Such as set forth in nucleotide position 83,580 to position 156,577 of SEQ ID NO: 13, 14 and 18.

Nucleic acid encoding polymorphic human AKAP10 proteins and genes  
5 provided herein may be isolated by screening suitable human cDNA or human genomic libraries under suitable hybridization conditions with nucleic acids such as those provided in SEQ. ID. NOS: 1, 3, 13, 14, 17 and 18. Suitable libraries can be prepared from human tissue and cell samples. In order to isolate cDNA encoding a polymorphic human AKAP10 it is preferable to screen libraries  
10 prepared from different tissues as the allele may not be expressed in all tissues or at similar levels in different tissues. The library can be screened with a portion of DNA including substantially the entire human AKAP10 or polymorphic AKAP10 protein-encoding sequence as set forth in SEQ. ID. NOS. 1, 3, 13, 14, 17 and 18, or the library may be screened with a suitable probe.

15 After screening the library, positive clones are identified by detecting a hybridization signal; the identified clones are characterized by restriction enzyme mapping and/or DNA sequence analysis, and then examined, by comparison with the sequences set forth herein to ascertain whether they include DNA encoding a complete polymorphic human AKAP10 protein (i.e., if they include translation  
20 initiation and termination codons). If the selected clones are incomplete, they may be used to rescreen the same or a different library to obtain overlapping clones. If the library is genomic, then the overlapping clones may include exons and introns. If the library is a cDNA library, then the overlapping clones will include an open reading frame. In both instances, clones may be identified by  
25 comparison with the DNA and encoded proteins provided herein.

In an alternative method, oligonucleotides based on the human AKAP10 or polymorphic AKAP10 protein-encoding sequence as set forth in SEQ. ID. NOS. 1, 3, 13, 14, 17 and 18, may be used to amplify fragments of the protein coding region of the AKAP10 gene from human cDNA or genomic sequence.

30 The isolated nucleic acid sequences can be incorporated into vectors for further manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either



expression or replication thereof. Selection and use of such vehicles are well within the skill of the artisan.

**D. Detection of Polymorphisms in Human AKAP10 Genes**

Methods of determining the presence or absence of allelic variants of a human AKAP10 gene are also provided. In particular methods, the detection or identification of a G, C, or T nucleotide at position 2073 of the sense strand of the human AKAP10 gene coding sequence (see SEQ ID NO: 1), or the detection or identification of a C, G or A nucleotide at the same position in the antisense strand of the human AKAP10 gene coding sequence, indicates the presence of an allelic variant. In these particular methods, the detection or identification of an A nucleotide at position 2073 of the sense strand of the human AKAP10 gene coding sequence, or the detection or identification of a T nucleotide at the same position in the antisense strand of the human AKAP10 gene coding sequence, indicates the absence of polymorphism.

Other methods for determining the presence or absence of an allelic variant of the AKAP10 gene detect or identify a nucleotide other than a C at position 83587 of the SEQ ID NO: 17 or a nucleotide other than a G on the complementary strand, a nucleotide other than a G at position 129600 of the SEQ ID NO: 17 or a nucleotide other than a C on the complementary strand or a nucleotide other than T at position 156,277 of SEQ ID NO: 17 or a nucleotide other than A on the complementary strand.

**1. Nucleic acid detection methods**

Generally, these methods are based in sequence-specific polynucleotides, oligonucleotides, probes and primers. Any method known to those of skill in the art for detecting a specific nucleotide within a nucleic acid sequence or for determining the identity of a specific nucleotide in a nucleic acid sequence is applicable to the methods of determining the presence or absence of an allelic variant of the AKAP10 gene. Such methods include, but are not limited to, techniques utilizing nucleic acid hybridization of sequence-specific probes, nucleic acid sequencing, selective amplification, analysis of restriction enzyme digests of the nucleic acid, cleavage of mismatched heteroduplexes of nucleic acid and probe, alterations of electrophoretic mobility, primer specific extension,

oligonucleotide ligation assay and single-stranded conformation polymorphism analysis. In particular, primer extension reactions that specifically terminate by incorporating a dideoxynucleotide are useful for detection. Several such general nucleic acid detection assays are known (see, *e.g.*, U.S. Patent No. 6,030,778).

5           a.       **Primer extension-based methods**

Several primer extension-based methods for determining the identity of a particular nucleotide in a nucleic acid sequence have been reported (see, *e.g.*, PCT Application Nos. PCT/US96/03651 (WO96/29431), PCT/US97/20444 (WO 98/20166), PCT/US97/20194 (WO 98/20019), PCT/US91/00046  
10 (WO91/13075), and U.S. Patent Nos. 5,547,835, 5,605,798, 5,622,824, 5,691,141, 5,872,003, 5,851,765, 5,856,092, 5,900,481, 6,043,031, 6,133,436 and 6,197,498.) In general, a primer is prepared that specifically hybridizes adjacent to a polymorphic site in a particular nucleic acid molecule. The primer is then extended in the presence of one or more dideoxynucleotides,  
15 typically with at least one of the dideoxynucleotides being the complement of the nucleotide that is polymorphic at the site. The primer and/or the dideoxynucleotides may be labeled to facilitate a determination of primer extension and identity of the extended nucleotide. In a preferred method, primer extension and/or the identity of the extended nucleotide(s) are determined  
20 by mass spectrometry (see, *e.g.*, PCT Application Nos. PCT/US96/03651 (WO96/29431), PCT Application No. PCT/US97/20444 (WO 98/20166), PCT Application No. PCT/US97/20194 (WO 98/20019), PCT Application No. PCT/US91/00046 (WO91/13075), and U.S. Patent Nos. 5,605,798, 5,622,824, 5,856,092.

25           b.       **Polymorphism-specific probe hybridization**

A preferred detection method is allele specific hybridization using probes overlapping the polymorphic site and having about 5, 10, 15, 20, 25, or 30 nucleotides around the polymorphic region. The probes can contain naturally occurring or modified nucleotides (see U.S. Patent No. 6,156,501). For  
30 example, oligonucleotide probes may be prepared in which the known polymorphic nucleotide is placed centrally (allele-specific probes) and then hybridized to target DNA under conditions which permit hybridization only if a

perfect match is found (Saiki *et al.* (1986) Nature 324:163; Saiki *et al.* (1989) Proc. Natl Acad. Sci USA 86:6230; and Wallace *et al.* (1979) Nucl. Acids Res. 6:3543). Such allele specific oligonucleotide hybridization techniques may be used for the simultaneous detection of several nucleotide changes in different polymorphic regions. For example, oligonucleotides having nucleotide sequences of specific allelic variants are attached to a hybridizing membrane and this membrane is then hybridized with labeled sample nucleic acid. Analysis of the hybridization signal will then reveal the identity of the nucleotides of the sample nucleic acid. In a preferred embodiment, several probes capable of hybridizing specifically to allelic variants are attached to a solid phase support, *e.g.*, a "chip". Oligonucleotides can be bound to a solid support by a variety of processes, including lithography. For example a chip can hold up to 250,000 oligonucleotides (GeneChip, Affymetrix, Santa Clara, CA). Mutation detection analysis using these chips comprising oligonucleotides, also termed "DNA probe arrays" is described *e.g.*, in Cronin *et al.* (1996) Human Mutation 7:244 and in Kozal *et al.* (1996) Nature Medicine 2:753. In one embodiment, a chip includes all the allelic variants of at least one polymorphic region of a gene. The solid phase support is then contacted with a test nucleic acid and hybridization to the specific probes is detected. Accordingly, the identity of numerous allelic variants of one or more genes can be identified in a simple hybridization experiment.

#### c. Nucleic acid amplification-based methods

In other detection methods, it is necessary to first amplify at least a portion of an AKAP gene prior to identifying the allelic variant. Amplification can be performed, *e.g.*, by PCR and/or LCR, according to methods known in the art. In one embodiment, genomic DNA of a cell is exposed to two PCR primers and amplification is performed for a number of cycles sufficient to produce the required amount of amplified DNA. In preferred embodiments, the primers are located between 150 and 350 base pairs apart.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J. C. *et al.*, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. *et al.*, 1989,

Proc. Natl. Acad. Sci. U.S.A. 86:1173-1177), Q-Beta Replicase (Lizardi, P. M. *et al.*, 1988, Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially  
5 useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

Alternatively, allele specific amplification technology, which depends on selective PCR amplification may be used in conjunction with the alleles provided herein. Oligonucleotides used as primers for specific amplification may carry the  
10 allelic variant of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) Nucleic Acids Res. 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) Tibtech 11:238; Newton *et al.* (1989) Nucl. Acids Res. 17:2503). In  
15 addition it may be desirable to introduce a restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) Mol. Cell Probes 6:1).

#### d. Nucleic acid sequencing-based methods

In one embodiment, any of a variety of sequencing reactions known in  
20 the art can be used to directly sequence at least a portion of an AKAP gene and to detect allelic variants, *e.g.*, mutations, by comparing the sequence of the sample sequence with the corresponding wild-type (control) sequence. Exemplary sequencing reactions include those based on techniques developed by Maxam and Gilbert (Proc. Natl. Acad. Sci. USA (1977) 74:560) or Sanger  
25 (Sanger *et al.* (1977) Proc. Natl. Acad. Sci 74:5463). It is also contemplated that any of a variety of automated sequencing procedures may be used when performing the subject assays (Biotechniques (1995) 19:448), including sequencing by mass spectrometry (see, for example, U.S. Patent Nos. 5,547,835, 5,691,141, and International PCT Application No.  
30 PCT/US94/00193 (WO 94/16101), entitled "DNA Sequencing by Mass Spectrometry" by H. Köster; U.S. Patent Nos. 5,547,835, 5,622,824, 5,851,765, 5,872,003, 6,074,823, 6,140,053 and International PCT

Application No. PCT/US94/02938 (WO 94/21822), entitled "DNA Sequencing by Mass Spectrometry Via Exonuclease Degradation" by H. Köster), and U.S. Pat. Nos. 5,605,798, 6,043,031, 6,197,498, and International Patent Application No. PCT/US96/03651 (WO 96/29431) entitled "DNA Diagnostics  
5 Based on Mass Spectrometry" by H. Köster; Cohen *et al.* (1996) Adv Chromatogr 36:127-162; and Griffin *et al.* (1993) Appl Biochem Biotechnol 38:147-159). It will be evident to one skilled in the art that, for certain embodiments, the occurrence of only one, two or three of the nucleic acid bases need be determined in the sequencing reaction. For instance, A-track  
10 sequencing or an equivalent, *e.g.*, where only one nucleotide is detected, can be carried out. Other sequencing methods are known (see, *e.g.*, in U.S. Patent No. 5,580,732 entitled "Method of DNA sequencing employing a mixed DNA-polymer chain probe" and U.S. Patent No. 5,571,676 entitled "Method for mismatch-directed *in vitro* DNA sequencing").

15                   e.       **Restriction enzyme digest analysis**

In some cases, the presence of a specific allele in nucleic acid, particularly DNA, from a subject can be shown by restriction enzyme analysis. For example, a specific nucleotide polymorphism can result in a nucleic molecule containing a restriction site which is absent from the nucleotide sequence of  
20 another allelic variant.

                  f.       **Mismatch Cleavage**

Protection from cleavage agents, such as, but not limited to, a nuclease, hydroxylamine or osmium tetroxide and with piperidine, can be used to detect mismatched bases in RNA/RNA DNA/DNA, or RNA/DNA heteroduplexes (Myers,  
25 *et al.* (1985) Science 230:1242). In general, the technique of "mismatch cleavage" starts by providing heteroduplexes formed by hybridizing a control nucleic acid molecule, which is optionally labeled, such as RNA or DNA, encoding an allelic variant, with a sample nucleic acid, such as RNA or DNA, obtained from a tissue sample. The double-stranded duplexes are treated with an  
30 agent, which cleaves single-stranded regions of the duplex such as duplexes formed based on basepair mismatches between the control and sample strands. For instance, RNA/DNA duplexes can be treated with RNase and DNA/DNA

hybrids treated with S1 nuclease to enzymatically digest the mismatched regions.

- In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine whether the control and sample nucleic acids have an identical nucleotide sequence or in which nucleotides they differ (see, for example, Cotton *et al.* (1988) Proc. Natl Acad Sci USA 85:4397; Saleeba *et al.* (1992) Methods Enzymol. 217:286-295). The control or sample nucleic acid is labeled for detection.

**g. Electrophoretic mobility alterations**

- In other embodiments, alteration in electrophoretic mobility is used to identify the type of allelic variant in an AKAP gene. For example, single-strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) Proc. Natl. Acad. Sci. USA 86:2766, see also Cotton (1993) Mutat Res 285:125-144; and Hayashi (1992) Genet Anal Tech Appl 9:73-79). Single-stranded DNA fragments of sample and control nucleic acids are denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In another preferred embodiment, the subject method uses heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) Trends Genet 7:5).

#### h. Polyacrylamide Gel Electrophoresis

In yet another embodiment, the identity of an allelic variant of a polymorphic region of an AKAP gene is obtained by analyzing the movement of a nucleic acid comprising the polymorphic region in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) Nature 313:495). When DGGE is used as the method of analysis, DNA will be modified to ensure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing agent gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) Biophys Chem 265:1275).

#### i. Oligonucleotide ligation assay (OLA)

In another embodiment, identification of the allelic variant is carried out using an oligonucleotide ligation assay (OLA), as described, *e.g.*, in U.S. Patent No. 4,998,617 and in Landegren, U. *et al.*, Science 241:1077-1080 (1988). The OLA protocol uses two oligonucleotides which are designed to be capable of hybridizing to abutting sequences of a single strand of a target. One of the oligonucleotides is linked to a separation marker, *e.g.*, biotinylated, and the other is detectably labeled. If the precise complementary sequence is found in a target molecule, the oligonucleotides will hybridize such that their termini abut, and create a ligation substrate. Ligation then permits the labeled oligonucleotide to be recovered using avidin, or another biotin ligand. Nickerson, D. A. *et al.* have described a nucleic acid detection assay that combines attributes of PCR and OLA (Nickerson, D. A. *et al.*, Proc. Natl. Acad. Sci. (U.S.A.) 87:8923-8927 (1990). In this method, PCR is used to achieve the exponential amplification of target DNA, which is then detected using OLA.

Several techniques based on this OLA method have been developed and can be used to detect specific allelic variants of a polymorphic region of a gene. For example, U.S. Pat. No. 5,593,826 discloses an OLA using an oligonucleotide having 3'-amino group and a 5'-phosphorylated oligonucleotide to form a conjugate having a phosphoramidate linkage. In another variation of OLA

described in Tobe *et al.* (1996) Nucl. Acids Res. 24: 3728), OLA combined with PCR permits typing of two alleles in a single microtiter well. By marking each of the allele-specific primers with a unique hapten, i.e. digoxigenin and fluorescein, each OLA reaction can be detected by using hapten specific antibodies that are

5 labeled with different enzyme reporters, alkaline phosphatase or horseradish peroxidase. This system permits the detection of the two alleles using a high throughput format that leads to the production of two different colors.

#### j. SNP detection methods

Also provided are methods for detecting single nucleotide polymorphisms.

10 Because single nucleotide polymorphisms constitute sites of variation flanked by regions of invariant sequence, their analysis requires no more than the determination of the identity of the single nucleotide present at the site of variation and it is unnecessary to determine a complete gene sequence for each patient. Several methods have been developed to facilitate the analysis of such single nucleotide

15 polymorphisms.

In one embodiment, the single base polymorphism can be detected by using a specialized exonuclease-resistant nucleotide, as disclosed, *e.g.*, in Mundy, C. R. (U.S. Patent No. 4,656,127). According to the method, a primer complementary to the allelic sequence immediately 3' to the polymorphic site is

20 permitted to hybridize to a target molecule obtained from a particular animal or human. If the polymorphic site on the target molecule contains a nucleotide that is complementary to the particular exonuclease-resistant nucleotide derivative present, then that derivative will be incorporated onto the end of the hybridized primer. Such incorporation renders the primer resistant to exonuclease, and

25 thereby permits its detection. Since the identity of the exonuclease-resistant derivative of the sample is known, a finding that the primer has become resistant to exonucleases reveals that the nucleotide present in the polymorphic site of the target molecule was complementary to that of the nucleotide derivative used in the reaction. This method has the advantage that it does not require the

30 determination of large amounts of extraneous sequence data.

In another embodiment, a solution-based method for determining the identity of the nucleotide of a polymorphic site is employed (Cohen, D. *et al.*



(French Patent 2,650,840; PCT Application No. WO91/02087)). As in the Mundy method of U.S. Patent No. 4,656,127, a primer is employed that is complementary to allelic sequences immediately 3' to a polymorphic site. The method determines the identity of the nucleotide of that site using labeled

5 dideoxynucleotide derivatives, which, if complementary to the nucleotide of the polymorphic site will become incorporated onto the terminus of the primer.

**k. Genetic Bit Analysis**

An alternative method, known as Genetic Bit Analysis or GBA™ is described by Goelet, *et al.* (U.S. Patent No. 6,004,744, PCT Application No.

10 92/15712). The method of Goelet, *et al.* uses mixtures of labeled terminators and a primer that is complementary to the sequence 3' to a polymorphic site. The labeled terminator that is incorporated is thus determined by, and complementary to, the nucleotide present in the polymorphic site of the target molecule being evaluated. In contrast to the method of Cohen *et al.* (French

15 Patent 2,650,840; PCT Application No. WO91/02087), the method of Goelet, *et al.* is preferably a heterogeneous phase assay, in which the primer or the target molecule is immobilized to a solid phase.

**l. Other primer-guided nucleotide incorporation procedures**

Other primer-guided nucleotide incorporation procedures for assaying

20 polymorphic sites in DNA have been described (Komher, J. S. *et al.*, Nucl. Acids Res. 17:7779-7784 (1989); Sokolov, B. P., Nucl. Acids Res. 18:3671 (1990); Syvanen, A. C., *et al.*, Genomics 8:684-692 (1990), Kuppuswamy, M. N. *et al.*, Proc. Natl. Acad. Sci. (U.S.A.) 88:1143-1147 (1991); Prezant, T. R. *et al.*, Hum. Mutat. 1:159-164 (1992); Ugozzoli, L. *et al.*, GATA 9:107-112 (1992); Nyren,

25 P. *et al.*, Anal. Biochem. 208:171-175 (1993)). These methods differ from GBA™ in that they all rely on the incorporation of labeled deoxynucleotides to discriminate between bases at a polymorphic site. In such a format, since the signal is proportional to the number of deoxynucleotides incorporated, polymorphisms that occur in runs of the same nucleotide can result in signals

30 that are proportional to the length of the run (Syvanen, A. C., *et al.*, Amer. J. Hum. Genet. 52:46-59 (1993)).

For determining the identity of the allelic variant of a polymorphic region located in the coding region of a gene, yet other methods than those described above can be used. For example, identification of an allelic variant which encodes a mutated protein can be performed by using an antibody specifically recognizing the mutant protein in, *e.g.*, immunohistochemistry or immunoprecipitation. Binding assays are known in the art and involve, *e.g.*, obtaining cells from a subject, and performing binding experiments with a labeled lipid, to determine whether binding to the mutated form of the protein differs from binding to the wild-type protein.

**10                    m.        Molecular structure determination**

If a polymorphic region is located in an exon, either in a coding or non-coding region of the gene, the identity of the allelic variant can be determined by determining the molecular structure of the mRNA, pre-mRNA, or cDNA. The molecular structure can be determined using any of the above described methods for determining the molecular structure of the genomic DNA, *e.g.*, sequencing and SSCP.

**n.        Mass spectrometric methods**

Nucleic acids can also be analyzed by detection methods and protocols, particularly those that rely on mass spectrometry (see, *e.g.*, U.S. Patent Nos. 5,605,798, 6,043,031, 6,197,498, and International Patent Application No. WO 96/29431, allowed co-pending U.S. Application Serial No. 08/617,256, allowed co-pending U.S. Application Serial No. 08/744,481, U.S. Application Serial No. 08/990,851, International PCT Application No. WO 98/20019). These methods can be automated (see, *e.g.*, co-pending U.S. Application Serial No. 09/285,481, which describes an automated process line). Preferred among the methods of analysis herein are those involving the primer oligo base extension (PROBE) reaction with mass spectrometry for detection (see *e.g.*, U.S. Patent Nos. 6,043,031 and 6,197,498, Patent Application Serial Nos. 09/287,681, 09/287,682, and 09/287,679, allowed co-pending U.S. Application Serial No. 08/744,481, International PCT Application No. PCT/US97/20444 (WO 98/20166), and based upon U.S. Patent Nos. 5,900,481, 6,024,925, 6,074,823, Application Serial Nos. 08/746,055, 08/786,988, 08/933,792,

08/746,055, and 08/786,988; see, also U.S. Application Serial No. 09/074,936, and published International PCT Application No. PCT/US97/20195 (WO 98/20020)).

5           A preferred format for performing the analyses is a chip based format in which the biopolymer is linked to a solid support, such as a silicon or silicon-coated substrate, preferably in the form of an array. More preferably, when analyses are performed using mass spectrometry, particularly MALDI, nanoliter volumes of sample are loaded on, such that the resulting spot is about, or  
10 smaller than, the size of the laser spot. It has been found that when this is achieved, the results from the mass spectrometric analysis are quantitative. The area under the peaks in the resulting mass spectra are proportional to concentration (when normalized and corrected for background). Methods for preparing and using such chips are described in U.S. Patent No. 6,024,925, co-  
15 pending U.S. Application Serial Nos. 08/786,988, 09/364,774, 09/371,150 and 09/297,575; see, also PCT Application No. PCT/US97/20195 (WO 98/20020). Chips and kits for performing these analyses are commercially available from SEQUENOM under the trademark MassARRAY™. MassARRAY™ relies on the fidelity of the enzymatic primer extension reactions combined with the  
20 miniaturized array and MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time of Flight) mass spectrometry to deliver results rapidly. It accurately distinguishes single base changes in the size of DNA fragments relating to genetic variants without tags.

          Multiplex methods allow for the simultaneous detection of more than one  
25 polymorphic region in a particular gene. This is the preferred method for carrying out haplotype analysis of allelic variants of the AKAP10 gene.

          Multiplexing can be achieved by several different methodologies. For example, several mutations can be simultaneously detected on one target sequence by employing corresponding detector (probe) molecules (*e.g.*,  
30 oligonucleotides or oligonucleotide mimetics). The molecular weight differences between the detector oligonucleotides must be large enough so that simultaneous detection (multiplexing) is possible. This can be achieved either by

the sequence itself (composition or length) or by the introduction of mass-modifying functionalities into the detector oligonucleotides (see below).

Mass modifying moieties can be attached, for instance, to either the 5'-end of the oligonucleotide, to the nucleobase (or bases), to the phosphate backbone, and to the 2'-position of the nucleoside (nucleosides) and/or to the terminal 3'-position. Examples of mass modifying moieties include, for example, a halogen, an azido, or of the type, XR, wherein X is a linking group and R is a mass-modifying functionality. The mass-modifying functionality can thus be used to introduce defined mass increments into the oligonucleotide molecule.

The mass-modifying functionality can be located at different positions within the nucleotide moiety (see, *e.g.*, U.S. Patent No. 5,547,835 and International PCT Application No. WO 94/21822). For example, the mass-modifying moiety, M, can be attached either to the nucleobase, (in case of the  $c^7$ -deazanucleosides also to C-7), to the triphosphate group at the alpha phosphate or to the 2'-position of the sugar ring of the nucleoside triphosphate. Modifications introduced at the phosphodiester bond, such as with alpha-thio nucleoside triphosphates, have the advantage that these modifications do not interfere with accurate Watson-Crick base-pairing and additionally allow for the one-step post-synthetic site-specific modification of the complete nucleic acid molecule *e.g.*, via alkylation reactions (see, *e.g.*, Nakamaye *et al.* (1988) Nucl. Acids Res. 16:9947-59). Particularly preferred mass-modifying functionalities are boron-modified nucleic acids since they are better incorporated into nucleic acids by polymerases (see, *e.g.*, Porter *et al.* (1995) Biochemistry 34:11963-11969; Hasan *et al.* (1996) Nucleic Acids Res. 24:2150-2157; Li *et al.* (1995) Nucl. Acids Res. 23:4495-4501).

Furthermore, the mass-modifying functionality can be added so as to affect chain termination, such as by attaching it to the 3'-position of the sugar ring in the nucleoside triphosphate. For those skilled in the art, it is clear that many combinations can be used in the methods provided herein. In the same way, those skilled in the art will recognize that chain-elongating nucleoside triphosphates can also be mass-modified in a similar fashion with numerous variations and combinations in functionality and attachment positions.

For example, without being bound to any particular theory, the mass-modification can be introduced for X in XR as well as using oligo-/polyethylene glycol derivatives for R. The mass-modifying increment (m) in this case is 44, i.e. five different mass-modified species can be generated by just changing m from 0 to 4 thus adding mass units of 45 (m=0), 89 (m=1), 133 (m=2), 177 (m=3) and 221 (m=4) to the nucleic acid molecule (*e.g.*, detector oligonucleotide (D) or the nucleoside triphosphates, respectively). The oligo/polyethylene glycols can also be monoalkylated by a lower alkyl such as, but are not limited to, methyl, ethyl, propyl, isopropyl and t-butyl. Other chemistries can be used in the mass-modified compounds (*see, e.g.*, those described in *Oligonucleotides and Analogues, A Practical Approach*, F. Eckstein, editor, IRL Press, Oxford, 1991).

In yet another embodiment, various mass-modifying functionalities, R, other than oligo/polyethylene glycols, can be selected and attached via appropriate linking chemistries, X. A simple mass-modification can be achieved by substituting H for halogens, such as F, Cl, Br and/or I, or pseudohalogens such as CN, SCN, NCS, or by using different alkyl, aryl or aralkyl moieties such as methyl, ethyl, propyl, isopropyl, t-butyl, hexyl, phenyl, substituted phenyl, benzyl, or functional groups such as  $\text{CH}_2\text{F}$ ,  $\text{CHF}_2$ ,  $\text{CF}_3$ ,  $\text{Si}(\text{CH}_3)_3$ ,  $\text{Si}(\text{CH}_3)_2(\text{C}_2\text{H}_5)$ ,  $\text{Si}(\text{CH}_3)(\text{C}_2\text{H}_5)_2$ ,  $\text{Si}(\text{C}_2\text{H}_5)_3$ . Yet another mass-modification can be obtained by attaching homo- or heteropeptides through the nucleic acid molecule (*e.g.*, detector (D)) or nucleoside triphosphates). One example, useful in generating mass-modified species with a mass increment of 57, is the attachment of oligoglycines (m) to nucleic acid molecules (r), *e.g.*, mass-modifications of 74 (r=1, m=0), 131 (r=1, m=1), 188 (r=1, m=2), 245 (r=1, m=3) are achieved. Simple oligoamides also can be used, *e.g.*, mass-modifications of 74 (r=1, m=0), 88 (r=2, m=0), 102 (r=3, m=0), 116 (r=4, m=0), *etc.* are obtainable. Variations in additions to those set forth herein will be apparent to the skilled artisan.

Different mass-modified detector oligonucleotides can be used to simultaneously detect all possible variants/mutants simultaneously. Alternatively, all four base permutations at the site of a mutation can be

detected by designing and positioning a detector oligonucleotide, so that it serves as a primer for a DNA/RNA polymerase with varying combinations of elongating and terminating nucleoside triphosphates. For example, mass modifications also can be incorporated during the amplification process.

- 5           A different multiplex detection format is one in which differentiation is accomplished by employing different specific capture sequences which are position-specifically immobilized on a flat surface (*e.g.*, a 'chip array'). If different target sequences T1-Tn are present, their target capture sites TCS1-TCSn will specifically interact with complementary immobilized capture
- 10 sequences C1-Cn. Detection is achieved by employing appropriately mass differentiated detector oligonucleotides D1-Dn, which are mass modifying functionalities M1-Mn.

**o. Other methods**

- Additional methods of analyzing nucleic acids include amplification- based
- 15 methods including polymerase chain reaction (PCR), ligase chain reaction (LCR), mini-PCR, rolling circle amplification, autocatalytic methods, such as those using QJ replicase, TAS, 3SR, and any other suitable method known to those of skill in the art.

- Other methods for analysis and identification and detection of
- 20 polymorphisms, include but are not limited to, allele specific probes, Southern analyses, and other such analyses.

**2. Primers, probes and antisense nucleic acid molecules**

- Primers refer to nucleic acids which are capable of specifically hybridizing to a nucleic acid sequence which is adjacent to a polymorphic region of interest
- 25 or to a polymorphic region and are extended. A primer can be used alone in a detection method, or a primer can be used together with at least one other primer or probe in a detection method. Primers can also be used to amplify at least a portion of a nucleic acid. For amplifying at least a portion of a nucleic acid, a forward primer (*i.e.*, 5' primer) and a reverse primer (*i.e.*, 3' primer) will
- 30 preferably be used. Forward and reverse primers hybridize to complementary stands of a double stranded nucleic acid, such that upon extension from each primer, a double stranded nucleic acid is amplified.

Probes refer to nucleic acids which hybridize to the region of interest and which are not further extended. For example, a probe is a nucleic acid which hybridizes adjacent to or at a polymorphic region of an AKAP gene and which by hybridization or absence of hybridization to the DNA of a subject will be

5 indicative of the identity of the allelic variant of the polymorphic region of the gene. Preferred probes have a number of nucleotides sufficient to allow specific hybridization to the target nucleotide sequence. Where the target nucleotide sequence is present in a large fragment of DNA, such as a genomic DNA

10 fragment of several tens or hundreds of kilobases, the size of a probe may have to be longer to provide sufficiently specific hybridization, as compared to a probe which is used to detect a target sequence which is present in a shorter fragment of DNA. For example, in some diagnostic methods, a portion of an AKAP gene may first be amplified and thus isolated from the rest of the chromosomal DNA and then hybridized to a probe. In such a situation, a shorter probe will likely

15 provide sufficient specificity of hybridization. For example, a probe having a nucleotide sequence of about 10 nucleotides may be sufficient.

Primers and probes (RNA, DNA (single-stranded or double-stranded), PNA and their analogs) described herein may be labeled with any detectable reporter or signal moiety including, but not limited to radioisotopes, enzymes, antigens,

20 antibodies, spectrophotometric reagents, chemiluminescent reagents, fluorescent and any other light producing chemicals. Additionally, these probes may be modified without changing the substance of their purpose by terminal addition of nucleotides designed to incorporate restriction sites or other useful sequences, proteins, signal generating ligands such as acridinium esters, and/or

25 paramagnetic particles.

These probes may also be modified by the addition of a capture moiety (including, but not limited to para-magnetic particles, biotin, fluorescein, dioxigenin, antigens, antibodies) or attached to the walls of microtiter trays to assist in the solid phase capture and purification of these probes and any DNA or

30 RNA hybridized to these probes. Fluorescein may be used as a signal moiety as well as a capture moiety, the latter by interacting with an anti-fluorescein antibody.

Any probe, primer or antisense molecule can be prepared according to methods well known in the art and described, *e.g.*, in Sambrook, J. Fritsch, E.F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. For example, discrete  
5 fragments of the DNA can be prepared and cloned using restriction enzymes. Alternatively, probes and primers can be prepared using the Polymerase Chain Reaction (PCR) using primers having an appropriate sequence.

Oligonucleotides may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially  
10 available from Biosearch (Novato, CA); Applied Biosystems (Foster City, CA) and other methods). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides, for example, can be prepared by use of controlled pore glass polymer supports (Sarin *et al.*, 1988, Proc. Natl. Acad. Sci.  
15 U.S.A. 85:7448-7451).

Probes and primers used in the methods of detecting allelic variants in human AKAP10 genes are of sufficient length to specifically hybridize to portions of AKAP10 gene at polymorphic sites. Typically such lengths depend upon the complexity of the source organism genome. For humans such lengths  
20 are at least 14-16 nucleotides, and typically may be 20, 30, 50, 100 or more nucleotides.

The methods of detecting polymorphisms in human AKAP10 genes provided herein, probes and primers include the following:

- (1) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or  
25 complement thereof, wherein nucleic acid molecule includes at least 5 contiguous nucleotides from nucleotide 2069 to nucleotide 2077 of SEQ. ID. NO: 3;
- (2) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid includes the nucleotide at position  
30 2073 of SEQ ID No. 1 replaced with G, C or T.
- (3) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5



contiguous nucleotides from nucleotide 129556 to nucleotide 129604 of SEQ. ID. NO: 14;

- (4) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid includes the nucleotide at position 5 129600 of SEQ ID No. 17 replaced with A, C or T;

(5) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5 contiguous nucleotides from nucleotide 83583 to nucleotide 83591 of SEQ. ID. NO: 13;

- 10 (6) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid includes the nucleotide at position 83587 of SEQ ID No. 17 replaced with G, A or T;

(7) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid molecule includes at least 5 15 contiguous nucleotides from nucleotide 156,273 to nucleotide 156281 of SEQ. ID. NO: 18;

(8) at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the nucleic acid includes the nucleotide at position 156277 of SEQ ID No. 17 replaced with C, A or G;

- 20 With respect to each of the above described probes and primers, they have fewer nucleotides than the sequence of nucleotides 138 to 2126 of SEQ. ID. NO: 1 or fewer nucleotides than the sequence of nucleotides 83,580 to 156,577 of SEQ ID NO: 17.

Antisense compounds may be conveniently and routinely made through 25 the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and 30 alkylated derivatives.

Antisense compounds are typically 8 to 30 nucleotides in length complementary to a targeted to a nucleic acid molecule and modulates its

expression. The targeted nucleic acid molecule represents the coding strand. For example, for the AKAP10-5 allelic variant an antisense compound is an antisense oligonucleotide that includes the complement of at least an 8 nucleotide segment of SEQ ID NO: 3 including the nucleotide at position 2073 of SEQ ID NO: 3.

An antisense compound can contain at least one modified nucleotide which can confer nuclease resistance or increase the binding of the antisense compound with the target nucleotide. The antisense compound can contain at least one internucleoside linkage wherein the modified internucleoside linkage of the antisense oligonucleotide can be a phosphorothioate linkage, a morpholino linkage or a peptide-nucleic acid linkage.

Preferred modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts.

Representative United States patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos.:

5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

An antisense compound can contain at least one modified sugar moiety wherein the modified sugar moiety of the antisense oligonucleotide is a 2'-O-methoxyethyl sugar moiety or a 2'-dimethylaminooxyethoxy sugar moiety.

Modified oligonucleotides may also contain one or more substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2' position: OH; F; O--, S--, or N-alkyl; O--, S--, or N-alkenyl; O--, S-- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C<sub>1</sub> to C<sub>10</sub> alkyl or C<sub>2</sub> to C<sub>10</sub> alkenyl and alkynyl. Particularly preferred are O[(CH<sub>2</sub>)<sub>n</sub> O]<sub>m</sub> CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub> OCH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub> NH<sub>2</sub>, O(CH<sub>2</sub>)<sub>n</sub> CH<sub>3</sub>, O(CH<sub>2</sub>)<sub>n</sub> NH<sub>2</sub>, and O(CH<sub>2</sub>)<sub>n</sub> ON[(CH<sub>2</sub>)<sub>n</sub> CH<sub>3</sub>]<sub>2</sub>, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2' position: C<sub>1</sub> to C<sub>10</sub> lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH<sub>3</sub>, OCN, Cl, Br, CN, CF<sub>3</sub>, OCF<sub>3</sub>, SOCH<sub>3</sub>, SO<sub>2</sub> CH<sub>3</sub>, ONO<sub>2</sub>, NO<sub>2</sub>, N<sub>3</sub>, NH<sub>2</sub>, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes an alkoxyalkoxy group, 2'-methoxyethoxy (2'-O-CH<sub>2</sub> CH<sub>2</sub> OCH<sub>3</sub>, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504). A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O(CH<sub>2</sub>)<sub>2</sub> ON(CH<sub>3</sub>)<sub>2</sub> group, also known as 2'-DMAOE.

Other preferred modifications include 2'-methoxy (2'-O-CH<sub>3</sub>), 2'-aminopropoxy (2'-OCH<sub>2</sub> CH<sub>2</sub> CH<sub>2</sub> NH<sub>2</sub>) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligonucleotides may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the reparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878;

5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,0531 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference.

- 5 An antisense compound can contain at least one modified nucleobase. Oligonucleotides may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified
- 10 nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and
- 15 cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines; 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-
- 20 deazaguanine and 3-deazaadenine.

- Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., Angewandte Chemie, International Edition,
- 25 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Crooke, S. T., and Lebleu, B. eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 289-302. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds. These include 5-
- 30 substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyl-adenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2.degree. C. (Sanghvi, Y. S., Crooke, S. T.

and Lebleu, B., eds., Antisense Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

The antisense compound can be a chimeric oligonucleotide.

- 5 Chimeric antisense compounds may be formed as composite structures of two or more oligonucleotides, modified oligonucleotides, oligonucleosides and/or oligonucleotide mimetics as described above. Such compounds have also been referred to in the art as hybrids or gapmers. Representative United States patents that teach the preparation of such hybrid structures include, but are not
- 10 limited to, U.S. Pat. Nos.: 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; and 5,700,922, each of which is herein incorporated by reference.

**E. Association of AKAP10 Allelic Variants with Morbidity or Increased Mortality**

- 15 Polymorphisms of the genome can lead to altered gene function, protein function or mRNA instability. To identify those polymorphisms that have clinical relevance is the goal of a world-wide scientific effort. Discovery of such polymorphisms will have a fundamental impact on the identification and development of diagnostics and drug discovery. The strategy to identify
- 20 valuable polymorphisms is cumbersome and dependent upon the availability of many large patient and control cohorts to show disease association. Furthermore, genes, and their associated polymorphisms, that cause a general risk of the population to suffer from any disease will escape these case/control studies entirely.

- 25 A morbidity susceptibility gene could be a gene that is expressed in many different cell types or tissues (housekeeping gene) and its altered function can facilitate the expression of a clinical phenotype caused by a disease-specific susceptibility gene that is involved in a pathway specific for this disorder. In other words, morbidity susceptibility genes might predispose people to develop a
- 30 distinct disease according to their genetic make-up for this disease. Candidates for these genes may involve basic cellular processes such as: transcription, translation, heat-shock proteins, protein trafficking, DNA repair, assembly

systems for subcellular structures (*e.g.*, mitochondria, peroxysomes and other cellular microbodies), receptor signaling cascades, immunology, etc. Those pathways control the quality of life at the cellular level as well as for the entire organism. Mutations/polymorphisms located in genes encoding proteins for

5 those pathways can reduce the fitness of cells and make the organism more susceptible to express the clinical phenotype caused by the action of a disease-specific susceptibility gene. Therefore, these morbidity susceptibility genes can be potentially involved in a whole variety of different complex diseases if not in all.

- 10 An example of possible candidate morbidity susceptibility genes are mutants of the A kinase anchoring protein (AKAP) genes. Protein phosphorylation is an important mechanism for enzyme regulation and signal transduction in eukaryotic cells. cAMP dependent protein kinase (PKA) mediates a variety of hormonal and neurotransmitter responses by phosphorylating a
- 15 wide variety of substrates including enzymes, membrane receptors, ion channels and transcription factors. AKAPs direct the subcellular localization of cAMP-dependent protein kinase by binding to its regulatory subunits and therefore plays role in G-protein mediated receptor-signalling pathways (see, *e.g.*, Huang *et al.* (1997) *Proc. Natl. Acad. Sci., USA* 94:11184). AKAPs have a PKA
- 20 binding region located in the COOH-terminal portion.

Polymorphic AKAP genes, such as those provided herein, serve as markers for detecting predisposition to disease and various conditions. Also, the AKAP alleles and gene products, especially the AKAP-10-5 gene product should be suitable pharmaceutical targets and gene therapy targets.

25 **F. Databases**

Use of databases containing sets of parameters associated with subjects in populations selected on the basis of apparent good health, not manifesting detectable disease (*i.e.*, an unbiased population not selected for any disease state), allows for identification of such morbidity susceptibility genes (see, U.S.

- 30 Provisional Application Serial No. 60/159,176 filed October 13, 1999, U.S. Provisional Application Serial No. 60/217,658 filed on July 10, 2000 and U.S. Application Serial No. 09/687,483 filed October 13, 2000).

For example, in a method for determining susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality in a human being, provided herein, exemplary steps include detecting the presence or absence of an allele of the human AKAP-10 containing other than an A at position 2073 of the coding sequence of the AKAP-10 gene; wherein the presence of an allele containing other than an A at position 2073 is indicative of increased susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality as compared to the susceptibility of a human being who does not comprise an allele containing other than an A at position 2073 of the AKAP-10 gene coding sequence.

As noted above, using a healthy patient database (see, U.S. Provisional Application Serial No. 60/159,176, U.S. Provisional Application Serial No. 60/217,658 and U.S. Application Serial No. 09/687,483 filed October 13, 2000), the frequency of occurrence of the AKAP10-5 SNP in such a population was found to decrease with age, thus making the allele a potential morbidity susceptibility gene, a gene associated with increased mortality or both. Using the healthy database, it was found that the homozygote GG genotype drops in the elderly population (over > 60 years), by a statistically significant amount,  $p=0.02$ .

#### G. Transgenic Animals

Methods for making transgenic animals using a variety of transgenes have been described in Wagner *et al.*, Proc. Nat. Acad. Sc. U.S.A., Vol. 78, p. 5016, 1981; Stewart *et al.*, Science, Vol. 217, p. 1046, 1982; Constantini *et al.*, Nature, Vol. 294, p. 92, 1981; Lacy *et al.*, Cell, Vol. 34, p. 343, 1983; McKnight *et al.*, Cell, Vol. 34, p. 335, 1983; Brinster *et al.*, Nature, Vol. 306, p. 332, 1983; Palmiter *et al.*, Nature, Vol. 300, p. 611, 1982; Palmiter *et al.*, Cell, Vol. 29, p. 701, 1982 and Palmiter *et al.*, Science, Vol. 222, p. 809, 1983. Such methods are described in U.S. Patent Nos. 6,175,057; 6,180,849 and 6,133,502.

The term "transgene" is used herein to describe genetic material that has been or is about to be artificially inserted into the genome of a mammalian cell, particularly a mammalian cell of a living animal. The transgene is used to

transform a cell, meaning that a permanent or transient genetic change, preferably a permanent genetic change, is induced in a cell following incorporation of exogenous DNA. A permanent genetic change is generally achieved by introduction of the DNA into the genome of the cell. Vectors for  
5 stable integration include, but are not limited to, plasmids, retroviruses and other animal viruses and YACS. Of interest are transgenic mammals, including, but are not limited to, cows, pigs, goats, horses and others, and particularly rodents, including rats and mice. Preferably, the transgenic-animals are mice.

Transgenic animals contain an exogenous nucleic acid sequence present  
10 as an extrachromosomal element or stably integrated in all or a portion of its cells, especially germ cells. Unless otherwise indicated, it will be assumed that a transgenic animal contains stable changes to the germline sequence. During the initial construction of the animal, "chimeras" or "chimeric animals" are generated, in which only a subset of cells have the altered genome. Chimeras  
15 are primarily used for breeding purposes in order to generate the desired transgenic animal. Animals having a heterozygous alteration are generated by breeding of chimeras. Male and female heterozygotes are typically bred to generate homozygous animals.

The exogenous gene is usually either from a different species than the  
20 animal host, or is otherwise altered in its coding or non-coding sequence. The introduced gene may be a wild-type gene, naturally occurring polymorphism or a genetically manipulated sequence, for example having deletions, substitutions or insertions in the coding or non-coding regions. When the introduced gene is a coding sequence, it is usually operably linked to a promoter, which may be  
25 constitutive or inducible, and other regulatory sequences required for expression in the host animal.

Transgenic animals can comprise other genetic alterations in addition to the presence of alleles of AKAP genes. For example, the genome can be altered to affect the function of the endogenous genes, contain marker genes, or  
30 contain other genetic alterations (*e.g.*, alleles of other genes associated with cardiovascular disease).



A "knock-out" of a gene means an alteration in the sequence of the gene that results in a decrease of function of the target gene, preferably such that target gene expression is undetectable or insignificant. A knock-out of an endogenous AKAP gene means that function of the gene has been substantially  
5 decreased so that expression is not detectable or only present at insignificant levels. "Knock-out" transgenics can be transgenic animals having a heterozygous knock-out of an AKAP gene or a homozygous knock-out. "Knock-outs" also include conditional knock-outs, where alteration of the target gene can occur upon, for example, exposure of the animal to a substance that promotes target  
10 gene alteration, introduction of an enzyme that promotes recombination at the target gene site (*e.g.*, Cre in the Cre-lox system), or other method for directing the target gene alteration postnatally.

A "knock-in" of a target gene means an alteration in a host cell genome that results in altered expression (*e.g.*, increased (including ectopic)) of the  
15 target gene, *e.g.*, by introduction of an additional copy of the target gene, or by operatively inserting a regulatory sequence that provides for enhanced expression of an endogenous copy of the target gene. "Knock-in" transgenics of interest can be transgenic animals having a knock-in of an AKAP gene. Such transgenics can be heterozygous or homozygous for the knock-in gene. "Knock-  
20 ins" also encompass conditional knock-ins.

A construct is suitable for use in the generation of transgenic animals if it allows the desired level of expression of an AKAP encoding sequence or the encoding sequence of another gene associated with cardiovascular disease. Methods of isolating and cloning a desired sequence, as well as suitable  
25 constructs for expression of a selected sequence in a host animal, are well known in the art and are described below.

For the introduction of a gene into the subject animal, it is generally advantageous to use the gene as a gene construct wherein the gene is ligated downstream of a promoter capable of and operably linked to expressing the gene  
30 in the subject animal cells. Specifically, a transgenic non-human mammal showing high expression of the desired gene can be created by microinjecting a vector ligated with said gene into a fertilized egg of the subject non-human

mammal (*e.g.*, rat fertilized egg) downstream of various promoters capable of expressing the protein and/or the corresponding protein derived from various mammals (rabbits, dogs, cats, guinea pigs, hamsters, rats, mice *etc.*, preferably rats *etc.*)

- 5            Useful vectors include Escherichia coli-derived plasmids, Bacillus subtilis-derived plasmids, yeast-derived plasmids, bacteriophages such as lambda, phage, retroviruses such as Moloney leukemia virus, and animal viruses such as vaccinia virus or baculovirus.

- Useful promoters for such gene expression regulation include, for  
10    example, promoters for genes derived from viruses (cytomegalovirus, Moloney leukemia virus, JC virus, breast cancer virus *etc.*), and promoters for genes derived from various mammals (humans, rabbits, dogs, cats, guinea pigs, hamsters, rats, mice *etc.*) and birds (chickens *etc.*) (*e.g.*, genes for albumin, insulin II, erythropoietin, endothelin, osteocalcin, muscular creatine kinase,  
15    platelet-derived growth factor beta, keratins K1, K10 and K14, collagen types I and II, atrial natriuretic factor, dopamine beta-hydroxylase, endothelial receptor tyrosine kinase (generally abbreviated Tie2), sodium-potassium adenosine triphosphorylase (generally abbreviated Na,K-ATPase), neurofilament light chain, metallothioneins I and IIA, metalloproteinase I tissue inhibitor, MHC class I  
20    antigen (generally abbreviated H-2L), smooth muscle alpha actin, polypeptide chain elongation factor 1 alpha (EF-1 alpha), beta actin, alpha and beta myosin heavy chains, myosin light chains 1 and 2, myelin base protein, serum amyloid component, myoglobin, renin *etc.*).

- It is preferable that the above-mentioned vectors have a sequence for  
25    terminating the transcription of the desired messenger RNA in the transgenic animal (generally referred to as terminator); for example, gene expression can be manipulated using a sequence with such function contained in various genes derived from viruses, mammals and birds. Preferably, the simian virus SV40 terminator *etc.* are commonly used. Additionally, for the purpose of increasing  
30    the expression of the desired gene, the splicing signal and enhancer region of each gene, a portion of the intron of a eukaryotic organism gene may be ligated

5' upstream of the promoter region, or between the promoter region and the translational region, or 3' downstream of the translational region as desired.

A translational region for a protein of interest can be obtained using the entire or portion of genomic DNA of blood, kidney or fibroblast origin from  
5 various mammals (humans, rabbits, dogs, cats, guinea pigs, hamsters, rats, mice *etc.*) or of various commercially available genomic DNA libraries, as a starting material, or using complementary DNA prepared by a known method from RNA of blood, kidney or fibroblast origin as a starting material. Also, an exogenous gene can be obtained using complementary DNA prepared by a known method  
10 from RNA of human fibroblast origin as a starting material. All these translational regions can be used in transgenic animals.

To obtain the translational region, it is possible to prepare DNA incorporating an exogenous gene encoding the protein of interest in which the gene is ligated downstream of the above-mentioned promoter (preferably  
15 upstream of the translation termination site) as a gene construct capable of being expressed in the transgenic animal.

DNA constructs for random integration need not include regions of homology to mediate recombination. Where homologous recombination is desired, the DNA constructs will comprise at least a portion of the target gene  
20 with the desired genetic modification, and will include regions of homology to the target locus. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown *et al.* (1990) Methods in  
25 Enzymology 185:527-537.

The transgenic animal can be created by introducing an AKAP gene construct into, for example, an unfertilized egg, a fertilized egg, a spermatozoon or a germinal cell containing a primordial germinal cell thereof, preferably in the embryogenic stage in the development of a non-human mammal (more preferably  
30 in the single-cell or fertilized cell stage and generally before the 8-cell phase), by standard means, such as the calcium phosphate method, the electric pulse method, the lipofection method, the agglutination method, the microinjection

method, the particle gun method, the DEAE-dextran method and other such method. Also, it is possible to introduce a desired AKAP gene into a somatic cell, a living organ, a tissue cell or other cell, by gene transformation methods, and use it for cell culture, tissue culture and any other method of propagation.

- 5 Furthermore, these cells may be fused with the above-described germinal cell by a commonly known cell fusion method to create a transgenic animal.

For embryonic stem (ES) cells, an ES cell line may be employed, or embryonic cells may be obtained freshly from a host, *e.g.* mouse, rat, guinea pig, *etc.* Such cells are grown on an appropriate fibroblast-feeder layer or grown  
10 in the presence of appropriate growth factors, such as leukemia inhibiting factor (LIF). When ES cells have been transformed, they may be used to produce transgenic animals. After transformation, the cells are plated onto a feeder layer in an appropriate medium. Cells containing the construct may be detected by employing a selective medium. After sufficient time for colonies to grow, they  
15 are picked and analyzed for the occurrence of homologous recombination or integration of the construct. Those colonies that are positive may then be used for embryo manipulation and blastocyst injection. Blastocysts are obtained from 4 to 6 week old superovulated females. The ES cells are trypsinized, and the modified cells are injected into the blastocoel of the blastocyst. After injection,  
20 the blastocysts are returned to each uterine horn of pseudopregnant females. Females are then allowed to go to term and the resulting litters screened for mutant cells having the construct. By providing for a different phenotype of the blastocyst and the ES cells, chimeric progeny can be readily detected. The chimeric animals are screened for the presence of the modified gene and males  
25 and females having the modification are mated to produce homozygous progeny. If the gene alterations cause lethality at some point in development, tissues or organs can be maintained as allogeneic or congenic grafts or transplants, or in *in vitro* culture.

- Animals containing more than one transgene, such as allelic variants of  
30 AKAP genes and/or other genes associated with morbidity and/or mortality can be made by sequentially introducing individual alleles into an animal in order to

produce the desired phenotype (manifestation of morbidity and/or predisposition to early mortality).

#### **H. Effect of Allelic Variants**

The effect of an allelic variant on a AKAP10 gene expression (amount of  
5 mRNA, mRNA stability) and AKAP protein (amount, stability, intracellular  
localization, activity) can be determined according to methods known in the art.  
Allelic variants of AKAP genes can be assayed individually or in combination.

In general, any method known to those skilled in the art of determining  
the presence or absence of a specific messenger RNA transcript or a specific  
10 translated protein can be used to presence or absence of a polymorphic protein  
or a polymorphism in the genetic sequence.

#### **1. RNA Analysis**

##### **A. Northern Blot Detection of RNA**

The northern blot technique is used to identify a RNA fragment of a  
15 specific size from a complex population of RNA using gel electrophoresis and  
nucleic acid hybridization. Northern blotting is a well-known technique in the art.  
Northern blot analysis is commonly used to detect specific RNA transcripts  
expressed in a variety of biological samples and have been described in  
Sambrook, J. et al. (Molecular Cloning, 3<sup>rd</sup> Edition, Cold Spring Harbor Press).

20 Briefly, total RNA is isolated from any biological sample by the method of  
Chomczynski and Sacchi (Anal. Biochem. (1987) 162, 156-159). Poly-  
adenylated mRNA is purified from total RNA using mini-oligo (dT) cellulose spin  
column kit with methods as outlined by the suppliers (Invitrogen, Carlsbad CA.).  
Denatured RNA is electrophoresed through a denaturing 1.5% agarose gel and  
25 transferred onto a nitrocellulose or nylon based matrix. The mRNA is detected  
by hybridization of a radiolabeled or biotinylated oligonucleotide probe specific to  
the polymorphic regions as disclosed herein.

##### **B. Dot Blot/Slot Blot**

Specific RNA transcripts can be detected using dot and slot blot assays  
30 to evaluate the presence of a specific nucleic acid sequence in a complex mix of  
nucleic acids. Specific RNA transcripts can be detected by adding the RNA  
mixture to a prepared nitrocellulose or nylon membrane. RNA is detected by the

hybridization of a radiolabeled or biotinylated oligonucleotide probe complementary to the AKAP sequences as disclosed herein.

### **C. RT-PCR**

The RT-PCR reaction may be performed, as described by K.-Q. Hu et al.,  
5 Virology 181:721-726 (1991), as follows: the extracted mRNA is transcribed in  
a reaction mixture 1 micromolar antisense primer, and 25 U AMV (avian  
myeloblastosis virus) or MMLV (Moloney murine leukemia virus) reverse  
transcriptase. Reverse transcription is performed and the cDNA is amplified in a  
PCR reaction volume with Taq polymerase. Optimal conditions for cDNA  
10 synthesis and thermal cycling can be readily determined by those skilled in  
the art.

### **2. Protein and Polypeptide Detection**

#### **Expression of Protein in a Cell Line**

Using the disclosed nucleic acids AKAP10 proteins may be expressed in a  
15 recombinantly engineered cell such as bacteria, yeast, insect, mammalian, or  
plant cells. Those of skill in the art are knowledgeable in the numerous  
expression systems available for expression of a nucleic acid encoding proteins  
such as polymorphic human AKAP10 proteins.

#### **A. Expression of AKAP Protein**

20 The isolated nucleic acid encoding a full-length polymorphic human  
AKAP10 protein, or a portion thereof, such as a fragment containing the site of  
the polymorphism, may be introduced into a vector for transfer into host cells.  
Fragments of the polymorphic human AKAP10 proteins can be produced by  
those skilled in the art, without undue experimentation, by eliminating portions  
25 of the coding sequence from the isolated nucleic acids encoding the full-length  
proteins.

Expression vectors are used expression of the protein in the host cell is  
desired. An expression vector includes vectors capable of expressing nucleic  
acids that are operatively linked with regulatory sequences, such as promoter  
30 regions, that are capable of effecting expression of such nucleic acids. Thus, an  
expression vector refers to a recombinant DNA or RNA construct, such as a  
plasmid, a phage, recombinant virus or other vector that, upon introduction into

an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome. Such

5 plasmids for expression of polymorphic human AKAP10-encoding nucleic acids in eukaryotic host cells, particularly mammalian cells, include cytomegalovirus (CMV) promoter-containing vectors, such as pCMV5, the pSV2dhfr expression vectors, which contain the SV40 early promoter, mouse dhfr gene, SV40 polyadenylation and splice sites and sequences necessary for maintaining the

10 vector in bacteria, and MMTV promoter-based vectors.

The nucleic acids encoding polymorphic human AKAP10 proteins, and vectors and cells containing the nucleic acids as provided herein permit production of the polymorphic proteins, as well as antibodies to the proteins. This provides a means to prepare synthetic or recombinant polymorphic human

15 AKAP10 proteins and fragments thereof that are substantially free of contamination from other AKAPs and proteins in general, the presence of which can interfere with analysis of the polymorphic proteins. In addition, the polymorphic proteins may be expressed in combination with selected other proteins that AKAP10 may associate with in cells. The ability to selectively

20 express the polymorphic AKAP10 proteins alone or in combination with other selected proteins makes it possible to observe the functioning of the recombinant polymorphic proteins within the environment of a cell. The expression of isolated nucleic acids encoding an AKAP protein will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter

25 (which is either constitutive or regulatable), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein. To obtain

30 high level expression of a cloned gene, it is desirable to construct expression vectors which contain, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator.

The skilled artisan recognizes that modifications can be made to an AKAP10 protein without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located purification sequences. Restriction sites or termination codons can also be introduced. There are expression vectors that specifically allow the expression of functional proteins.

One such vector, Plasmid 577, described in U.S. Pat. No. 6,020,122 and incorporated herein by reference, has been constructed for the expression of secreted antigens in a permanent cell line. This plasmid contains the following DNA segments: (a) a fragment of pBR322 containing bacterial beta-lactamase and origin of DNA replication; (b) a cassette directing expression of a neomycin resistance gene under control of HSV-1 thymidine kinase promoter and poly-A addition signals; (c) a cassette directing expression of a dihydrofolate reductase gene under the control of a SV-40 promoter and poly-A addition signals; (d) cassette directing expression of a rabbit immunoglobulin heavy chain signal sequence fused to a modified hepatitis C virus (HCV) E2 protein under the control of the Simian Virus 40 T-Ag promoter and transcription enhancer, the hepatitis B virus surface antigen (HBsAg) enhancer I followed by a fragment of Herpes Simplex Virus-1 (HSV-1) genome providing poly-A addition signals; and (e) a fragment of Simian Virus 40 genome late region of no function in this plasmid. All of the segments of the vector were assembled by standard methods known to those skilled in the art of molecular biology. Plasmids for the expression of secreted AKAP proteins can be constructed by replacing the hepatitis C virus E2 protein coding sequence in plasmid 577 with a AKAP sequence of SEQ ID NO: 3 or a fragment thereof. The resulting plasmid is transfected into CHO/dhfr-cells (DXB-111) (Uriacio, et al., PNAS 77, 4451-4466; 1980); these cells are available from the A.T.C.C., 12301 Parklawn Drive, Rockville, Md. 20852, under Accession No. CRL 9096), using the cationic



liposome-mediated procedure (P. L. Felgner et al., PNAS 84:7413-7417 (1987)). Proteins are secreted into the cell culture media.

Incorporation of cloned DNA into a suitable expression vector, transfection of cells with a plasmid vector or a combination of plasmid vectors, each encoding one or more distinct proteins or with linear DNA, and selection of transfected cells are well known in the art (see, e.g., Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press). Heterologous nucleic acid may be introduced into host cells by any method known to those of skill in the art, such as transfection with a vector encoding the heterologous nucleic acid by  $\text{CaPO}_4$  precipitation (see, e.g., Wigler et al. (1979) *Proc. Natl. Acad. Sci. USA* 76:1373-1376) or lipofectamine (GIBCO BRL #18324-012). Recombinant cells can then be cultured under conditions whereby the polymorphic human AKAP10 protein encoded by the nucleic acid is expressed. Suitable host cells include mammalian cells (e.g., HEK293, including but are not limited to, those described in U.S. Patent No. 5,024,939 to Gorman (see, also, Stillman et al. (1985) *Mol. Cell. Biol.* 5:2051-2060); also, HEK293 cells available from ATCC under accession #CRL 1573), CHO, COS, BHKBI and Ltk<sup>-</sup> cells, mouse monocyte macrophage P388D1 and J774A-1 cells (available from ATCC, Rockville, MD) and others known to those of skill in this art), yeast cells, including, but are not limited to, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha*, human cells and bacterial cells, including, but are not limited to, *Escherichia coli*. *Xenopus* oocytes may also be used for expression of *in vitro* RNA transcripts of the DNA.

Heterologous nucleic acid may be stably incorporated into cells or may be transiently expressed using methods known in the art. Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene (such as, for example, the gene for thymidine kinase, dihydrofolate reductase, neomycin resistance, and the like), and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene (such as the *E. coli*  $\beta$ -galactosidase gene) to monitor transfection

efficiency. Selectable marker genes are not included in the transient transfections because the transfectants are typically not grown under selective conditions, and are usually analyzed within a few days after transfection.

- Heterologous nucleic acid may be maintained in the cell as an episomal element or may be integrated into chromosomal DNA of the cell. The resulting recombinant cells may then be cultured or subcultured (or passaged, in the case of mammalian cells) from such a culture or a subculture thereof. Methods for transfection, injection and culturing recombinant cells are known to the skilled artisan. Similarly, the polymorphic human AKAP10 proteins or fragments thereof may be purified using protein purification methods known to those of skill in the art. For example, antibodies or other ligands that specifically bind to the proteins may be used for affinity purification and immunoprecipitation of the proteins.

#### **B. Protein Purification**

- The AKAP10 proteins may be purified by standard techniques well known to those of skill in the art. Recombinantly produced proteins can be directly expressed or expressed as a fusion protein. The recombinant protein is purified by a combination of cell lysis (e.g., sonication, French press) and affinity chromatography. The proteins, recombinant or synthetic, may be purified to substantial purity by standard techniques well known in the art, including detergent solubilization, selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. (See, for example, R. Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag: New York (1982); Deutscher, *Guide to Protein Purification*, Academic Press (1990)). For example, antibodies may be raised to the proteins as described herein. Purification from *E. coli* can be achieved following procedures described in U.S. Pat. No. 4,511,503. The protein may then be isolated from cells expressing the protein and further purified by standard protein chemistry techniques as described herein. Detection of the expressed protein is achieved by methods known in the art and include, for example, radioimmunoassays, Western blotting techniques or immunoprecipitation.

### 3. Immunodetection of the AKAP10 protein.

Generally, the AKAP proteins, when presented as an immunogen, should elicit production of a specifically reactive antibody. Immunoassays for determining binding are well known to those of skill in the art, as are methods of making and assaying for antibody binding specificity/affinity. Exemplary immunoassay formats include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays, in vivo expression or immunization protocols with purified protein preparations. In general, the detection of immunocomplex formation is well known in the art and may be achieved by methods generally based upon the detection of a label or marker, such as any of the radioactive, fluorescent, biological or enzymatic tags. Labels are well known to those skilled in the art (see U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference). Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody or a biotin/avidin ligand binding arrangement, as is known in the art.

#### A. Production of Polyclonal Antisera Against AKAP

Antibodies can be raised to AKAP proteins, including individual, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these proteins in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. A variety of analytic methods are available to generate a hydrophilicity profile of proteins. Such methods can be used to guide the artisan in the selection of peptides for use in the generation or selection of antibodies which are specifically reactive, under immunogenic conditions. See, e.g., J. Janin, *Nature*, 277 (1979) 491-492; Wolfenden, et al., *Biochemistry* 20(1981) 849-855; Kyte and Doolite, *J. Mol. Biol.* 157 (1982) 105-132; Rose, et al., *Science* 229 (1985) 834-838.

A number of immunogens can be used to produce antibodies specifically reactive with AKAP proteins. An isolated recombinant, synthetic, or native polynucleotide are the preferred immunogens (antigen) for the production of monoclonal or polyclonal antibodies. Polypeptides are typically denatured, and

optionally reduced, prior to formation of antibodies for screening expression libraries or other assays in which a putative AKAP protein is expressed or denatured in a non-native secondary, tertiary, or quaternary structure.

The AKAP protein (SEQ ID NO: 4, or a portion thereof) is injected into an  
5 animal capable of producing antibodies. Either monoclonal or polyclonal  
antibodies can be generated for subsequent use in immunoassays to measure  
the presence and quantity of the protein. Methods of producing polyclonal  
antibodies are known to those of skill in the art. In brief, an immunogen  
(antigen), preferably a purified protein, a protein coupled to an appropriate carrier  
10 (e.g., GST, keyhole limpet hemanocyanin, etc.), or a protein incorporated into an  
immunization vector such as a recombinant vaccinia virus (see, U.S. Pat. No.  
4,722,848) is mixed with an adjuvant and animals are immunized with the  
mixture. The animal's immune response to the immunogen preparation is  
monitored by taking test bleeds and determining the titer of reactivity to the  
15 protein of interest. When appropriately high titers of antibody to the immunogen  
are obtained, blood is collected from the animal and antisera are prepared.  
Further fractionation of the antisera to enrich for antibodies reactive to the  
protein is performed where desired (See, e.g., Coligan, Current Protocols in  
Immunology, Wiley/Greene, NY (1991); and Harlow and Lane, Antibodies: A  
20 Laboratory Manual, Cold Spring Harbor Press, NY (1989)).

#### B. Western Blotting of Tissue Samples for the AKAP protein

Biological samples are homogenized in SDS-PAGE sample buffer (50 mM  
Tris-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10%  
glycerol), heated at 100 degrees Celsius for 10 min and run on a 14% SDS-  
25 PAGE with a 25 mM Tris-HCl, pH 8.3, 250 mM Glycine, 0.1% SDS running  
buffer. The proteins are electrophoretically transferred to nitrocellulose in a  
transfer buffer containing 39 mM glycine, 48 mM Tris-HCl, pH 8.3, 0.037%  
SDS, 20% methanol. The nitrocellulose is dried at room temperature for 60 min  
and then blocked with a PBS solution containing either bovine serum albumin or  
30 5% nonfat dried milk for 2 hours at 4 degrees Celsius.

The filter is placed in a heat-sealable plastic bag containing a solution of  
5% nonfat dried milk in PBS with a 1:100 to 1:2000 dilution of affinity purified

- anti-AKAP peptide antibodies, incubated at 4 degrees Celsius for 2 hours, followed by three 10 min washes in PBS. An alkaline phosphatase conjugated secondary antibody (i.e., anti-mouse/rabbit IgG), is added at a 1:200 to 1:2000 dilution to the filter in a 150 mM NaCl, 50 mM Tris-HCl, pH 7.5 buffer and
- 5 incubated for 1 h at room temperature.

- The bands are visualized upon the addition and development of a chromogenic substrate such as 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT). The filter is incubated in the solution at room temperature until the bands develop to the desired intensity. Molecular mass
- 10 determination is made based upon the mobility of pre-stained molecular weight standards (Rainbow markers, Amersham, Arlington Heights, Ill.).

#### C. Microparticle Enzyme Immunoassay (MEIA)

- AKAP10 proteins and peptides are detected using a standard commercialized antigen competition EIA assay or polyclonal antibody sandwich
- 15 EIA assay on the IMx.RTM Analyzer (Abbott Laboratories, Abbott Park, Ill.). Samples containing the AKAP10 protein are incubated in the presence of anti-AKAP10 coated microparticles. The microparticles are washed and secondary polyclonal anti-AKAP10 antibodies conjugated with detectable entities (i.e., alkaline phosphatase) are added and incubated with the microparticles. The
- 20 microparticles are washed and the bound antibody/antigen/antibody complexes are detected by adding a substrate (i.e. 4-methyl umbelliferyl phosphate) (MUP) that will react with the secondary conjugated antibody to generate a detectable signal.

#### D. Immunocytochemistry

- 25 Intracellular localization of the AKAP10 protein can be determined by a variety of in situ hybridization techniques. In one method cells are fixed with fixed in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS; pH7.4) for 5 min., rinsed in PBS for 2 min., dilapidated and dehydrated in an ethanol series (50, 70 and 95%) (5 min. each and stored in 95% ethanol at 4 degrees
- 30 Celsius).

The cells are stained with the primary anti-AKAP10 antibody and a mixture of secondary antibodies used for detection. Laser-scanning confocal microscopy is performed to localize the AKAP10 protein.

#### 4. Binding Assays

- 5            Assays to measure the interaction between AKAP10 and the regulatory subunits RI and/or RII of the Protein Kinase A holoenzyme include immobilized binding assays, solution binding assays and the like. In some instances, it may be desirable to monitor binding between AKAP10 and PKA. In other instances, it may be desirable to specifically monitor the binding between AKAP10 and a
- 10 cellular component (other than PKA) to which it binds. Assays may be performed in a variety of formats, including cell-based assays, such as di-hybrid screening or complementation assays as described in U.S. Pat. No. 5,283,173 and Patent Cooperation Treaty (PCT) Publication No. WO 91/16457, respectively. Assays of this type are particularly useful for assessing
- 15 intracellular efficacy of test compounds. Non-cell-based assays include scintillation proximity assays, cAMP competition assays, ELISA assays, radioimmunoassays, chemiluminescent assays, and the like. Such assay procedures are well known in the art and generally described, e.g., in Boudet et al., J. Immunol. Meth., 142:73-82 (1991); Ngai et al., J. Immunol. Meth.,
- 20 158:267-276 (1993); Pruslin et al., J. Immunol. Meth., 137:27-35 (1991); Udenfriend et al., Proc. Natl. Acad. Sci. USA, 82:8672-8676 (1985); Udenfriend et al., Anal. Biochem., 161:494-500 (1987); Bosworth and Towers, Nature, 341:167-168 (1989); Gilman, Proc. Natl. Acad. Sci. USA, 67:305-312 (1970); and U.S. Pat. No. 4,568,649.

#### 25            A. In vitro binding assay

- Huang et al. Proc. Natl. Acad. Sci. USA, 272:8057-8064 (1997); Protein preparations containing AKAP10 are incubated with glutathione resin in PBS for 2 hours at 4 degrees Celsius with 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 5 mM benzamidine, and 5 mM  $\beta$ -
- 30 mercapthoethanol and washed extensively with the same buffer. 200 micrograms of PKA regulatory subunit RII and/or RI were added to the resin and incubated at 4 degrees Celsius. Proteins associated with the AKAP10 are eluted

and analyzed by Laemmli electrophoresis. The proteins were visualized by Coomassie Staining. PKA proteins can be radiolabeled or labeled with a flurophore to allow detection.

B. PKA phosphorylation of protein substrate

5       Cyclic AMP-dependent protein kinase (PKA) catalyzes the transfer of gamma phosphate from adenosine triphosphate (ATP) to a serine or threonine residue in a protein substrate. A short synthetic peptide (Leucine-Arg-Arg-Alanine-Serine-Leucine-Glycine or LRRASLG) is used as a substrate to assay the specific type of PKA activity as described in Pearson et.  
10 al., Methods of Enzymology 200, 62-81 (1991).

The PKA assay is typically carried out in a reaction of the enzyme with a peptide substrate and gamma  $^{32}\text{P}$ -ATP followed by separation of the  $^{32}\text{P}$ -peptide product from the unreacted gamma  $^{32}\text{P}$ -ATP on a phosphocellulose membrane. This method requires at least one basic amino acid residue in the peptide  
15 substrate. The peptide substrate can be tagged with a biotin group so that the biotinylated  $^{32}\text{P}$ -peptide product consistently binds to a streptavidin membrane in a manner independent of the peptide sequence as described in Goueli et al Analytical Biochemistry 225, 10-17, (1995). The separation of the  $^{32}\text{P}$ -peptide product from the free gamma  $^{32}\text{P}$ -ATP using affinity binding and ultrafiltration  
20 separation to analyze a mixture sample as described in U.S. Patent No. 5,869,275.

If the mutation is located in an intron, the effect of the mutation can be determined, *e.g.*, by producing transgenic animals in which the allelic variant has been introduced and in which the wild-type gene or predominant allele may have  
25 been knocked out. Comparison of the level of expression of the protein in the mice transgenic for the allelic variant with mice transgenic for the predominant allele will reveal whether the mutation results in increased or decreased synthesis of the associated protein and/or aberrant tissue distribution or intracellular localization of the associated protein. Such analysis could also be  
30 performed in cultured cells, in which the human variant allele gene is introduced and, *e.g.*, replaces the endogenous gene in the cell. For mutant AKAP proteins binding to signalling enzymes such as PKA is also examined. Thus, depending

on the effect of the alteration a specific treatment can be administered to a subject having such a mutation. Accordingly, if the mutation results in decreased production of AKAP protein, the subject can be treated by administration of a compound which increases synthesis, such as by increasing  
5 AKAP gene expression, and wherein the compound acts at a regulatory element different from the one which is mutated. Alternatively, if the mutation results in increased AKAP protein, the subject can be treated by administration of a compound which reduces protein production, *e.g.*, by reducing AKAP gene expression or a compound which inhibits or reduces the activity of AKAP  
10 protein.

**I. Diagnostic and Prognostic Assays**

Typically, an individual allelic variant that associates with morbidity and/or mortality and/or an alteration in signal transduction will not be used in isolation as a prognosticator. An allelic variant typically will be one of a plurality  
15 of indicators that are used. The other indicators may be the manifestation of other risk factors for morbidity and/or mortality and other evidence of altered signal transduction.

Useful combinations of allelic variants of the AKAP10 gene can be determined. Variants can be assayed individually or assayed simultaneously  
20 using multiplexing methods as described above or any other labelling method that allows different variants to be identified. In particular, variants of the AKAP10 gene may be assayed using kits (see below) or any of a variety of microarrays known to those in the art. For example, oligonucleotide probes comprising the polymorphic regions surrounding any polymorphism in the  
25 AKAP10 gene may be designed and fabricated using methods such as those described in U.S. Patent Nos. 5,492,806; 5,525,464; 5,695,940; 6,018,041; 6,025,136; WO 98/30883; WO 98/56954; WO99/09218; WO 00/58516; WO 00/58519, or references cited therein.

**J. Screening assays for modulators**

30 Modulators of AKAP10 biological activities may be identified by using any of the disclosed methods related to AKAP10 binding to PKA, AKAP10



localization in the mitochondria, binding to other signaling enzymes and phosphorylation by PKA.

In particular, once a variant protein such as AKAP10-5 is contacted with a potential modulating molecule the effect of the molecule on the binding  
5 between AKAP protein and PKA can be determined by using the assays disclosed in the section entitled "Effect of Allelic Variants". For example mitochondria can be isolated from cells exposed to the potential modulating molecule. PKA protein can then be isolated and quantitated or phosphorylation can be determined using the disclosed PKA assay. An increase in the amount of  
10 PKA protein in the mitochondria or the quantity of test peptide phosphorylated by mitochondrial isolated PKA would indicate a positive effect of the test molecule. Binding of AKAP10 protein and PKA could be directly assessed using an in vitro binding assay, or other disclosed binding assays, or by immunoassays such as immunoprecipitation.

15 For allelic variants that do not alter the AKAP10 protein the effect of a potential modulating molecule can be assayed by examining PKA RNA using the various methods disclosed for RNA analysis.

#### K. Ribozymes

A ribozyme targets the RNA genome and RNA transcripts and copies  
20 thereof. Each ribozyme molecule contains a catalytically active segment capable of cleaving the plus or minus strand of RNA, and further contains flanking sequences having a nucleotide sequence complementary to portions of the target RNA. The flanking sequences serve to anneal the ribozyme to the RNA in a site-specific manner. Absolute complementarity of the flanking sequences to the  
25 target sequence is not necessary, however, as only an amount of complementarity sufficient to form a duplex with the target RNA and to allow the catalytically active segment of the ribozyme to cleave at the target sites is necessary. Thus, only sufficient complementarity to permit the ribozyme to be hybridizable with the target RNA is required.

30 Enzymatic RNA molecule can be formed in a hammerhead motif, but the ribozyme may also be formed in the motif of a hairpin, hepatitis delta virus, group I intron or RNase P RNA (in association with an RNA guide sequence).

Examples of hammerhead motifs are described by Rossi et al., AIDS Res. Hum. Retrovir. 8:183 (1992), hairpin motifs are described by Hampel et al., Biochem. 28:4929 (1989) and Hampel et al., Nucl. Acids Res. 18:299 (1990), the hepatitis delta virus motif is exemplified in Perrotta and Been, Biochem. 31:16  
5 (1992), an RNaseP motif is described in Gueerier-Takada et al., Cell 35:849 (1983), and examples of the group I intron motif are described in Cech et al., U.S. Pat. No. 4,987,071, each of the foregoing disclosures being incorporated herein by reference.

Ribozymes may be prepared by chemical synthesis or produced by  
10 recombinant vectors according to methods established for the synthesis of RNA molecules. See, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), incorporated herein by reference. The ribozyme sequence may be synthesized, for example, using RNA polymerases such as T7 or SP6. The ribozymes may be  
15 prepared from a corresponding DNA sequence (DNA which on transcription yields a ribozyme) operably linked to an RNA polymerase promoter such as the promoter for T7 RNA polymerase or SP6 RNA polymerase. A DNA sequence corresponding to a ribozyme may be ligated in to a DNA vector, such as a plasmid, bacteriophage or other virus. Where the transfer vector contains an  
20 RNA polymerase promoter operably linked to DNA corresponding to a ribozyme, the ribozyme may be conveniently produced upon incubation with an RNA polymerase. Ribozymes may therefore be produced in vitro by incubation of RNA polymerase with an RNA polymerase promoter operably linked to DNA corresponding to a ribozyme, in the presence of ribonucleotides. *In vivo*,  
25 prokaryotic or eukaryotic cells, including, but are not limited to, mammalian cells, can be transfected with an appropriate vector containing genetic material corresponding to a ribozyme, operably linked to an RNA polymerase promoter such that the ribozyme is transcribed in the host cell. Ribozymes may be directly transcribed in vivo from a transfer vector, or alternatively, may be transcribed as  
30 part of a larger RNA molecule. For example, DNA corresponding to ribozyme sequence may be ligated into the 3' end of a carrier gene, for example, after a translation stop signal. Larger RNA molecules may help to stabilize the ribozyme

molecules against nuclease digestion within the cells. On translation the carrier gene may give rise to a protein, whose presence can be directly assayed if desired, for example, by enzymatic reaction when the carrier gene encodes an enzyme.

- 5           Those of skill in the art based on the above description and the sequences disclosed herein can design ribozymes to target RNA representing the allelic variants of the AKAP10 gene. For example, the sequence of anti-AKAP10-5 hammerhead ribozyme is
- 10           5- U G C A C U G A N G A G C C U G G A C G A A A C U – 3' (SEQ ID NO: 25). The sequence UGCA is complementary to target RNA with C hybridizing to the G nucleotide at position 2073 of SEQ ID NO: 3 of the AKAP10-5 allelic variant. The simplest hammerhead ribozyme must have UG at the 5' end of the substrate binding site.

#### L. Kits

- 15           Kits can be used to indicate whether a subject is at risk of increased susceptibility to morbidity and/or predisposition for premature or increased or early mortality. The kits can also be used to determine if a subject has a genetic predisposition to a disorder related to signal transduction. This information could be used, *e.g.*, to optimize treatment of such individuals as a particular genotype
- 20           may be associated with drug response.

- The kits comprise a probe or primer which is capable of hybridizing adjacent to or at a polymorphic region of AKAP10 and thereby identifying whether the AKAP10 gene contains an allelic variant which is associated with increased susceptibility to morbidity and/or predisposition for premature or
- 25           increased or early mortality or a genetic predisposition to a disorder related to signal transduction and/or protein phosphorylation. The kits further comprise instructions for use in carrying out assays, interpreting results and diagnosing a subject as having increased susceptibility to morbidity and/or predisposition for premature or increased or early mortality or a genetic predisposition to a disorder
- 30           related to signal transduction and/or protein phosphorylation.

          Kits for amplifying a region of AKAP10 gene or other genes associated with morbidity and/or mortality and/or signal transduction comprise two primers

which flank a polymorphic region of the gene of interest. For example primers can comprise the sequences of SEQ ID NOs: 5, 6, 7, 10, 12 and 16. For other assays, primers or probes hybridize to a polymorphic region or 5' or 3' to a polymorphic region depending on which strand of the target nucleic acid is used.

- 5 For example, specific probes and primers comprise sequences designated as SEQ ID NOs: 8, 15, 19 and 20. Those of skill in the art can synthesize primers and probes which hybridize adjacent to or at the polymorphic regions described herein and other SNPs in genes associated with morbidity and/or mortality and/or signal transduction

- 10 Yet other kits comprise at least one reagent necessary to perform an assay. For example, the kit can comprise an enzyme, such as a nucleic acid polymerase. Alternatively the kit can comprise a buffer or any other necessary reagent.

- Yet other kits comprise microarrays of probes to detect allelic variants of  
15 the AKAP10 gene. The kits further comprise instructions for their use and interpreting the results.

- The practice of methods and development of the products provided herein employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA,  
20 and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D.N. Glover ed., 1985); Oligonucleotide Synthesis (M.J. Gait ed., 1984); Mullis *et al.* U.S. Patent  
25 No. 4,683,195; Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Culture of Animal Cells (R.I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc.,  
30 New York); Gene Transfer Vectors For Mammalian Cells (J.H. Miller and M.P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu *et al.* eds., Immunochemical Methods In Cell and

Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook of Experimental Immunology, Volumes I-IV (D.M. Weir and C.C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

- 5        The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

#### EXAMPLE 1

##### Isolation of DNA from blood samples of a healthy donor population

- Healthy samples were obtained through the blood bank of San
- 10    Bernardino, CA. Both parents of the blood donors were of Caucasian origin. Practically a healthy subject, when human, is defined as human donor who passes blood bank criteria to donate blood for eventual use in the general population. These criteria are as follows: free of detectable viral, bacterial, mycoplasma, and parasitic infections; not anemic; and then further selected
- 15    based upon a questionnaire regarding history. Thus, a healthy population represents an unbiased population of sufficient health to donate blood according to blood bank criteria, and not further selected for any disease state. Typically, such individuals are not taking any medications.

- Blood was obtained from a donor by venous puncture and preserved with
- 20    1 mM EDTA pH 8.0. Ten milliliters of whole blood from each donor was centrifuged at 2000 x g. One milliliter of the buffy coat was added to 9 milliliters of 155 mM  $\text{NH}_4\text{Cl}$ , 10 mM  $\text{KHCO}_3$ , and 0.1 mM  $\text{Na}_2\text{EDTA}$ , incubated 10 minutes at room temperature and centrifuged for 10 minutes at 2000x g. The supernatant was removed, and the white cell pellet was washed in 155 mM
- 25     $\text{NH}_4\text{Cl}$ , 10 mM  $\text{KHCO}_3$ , and 0.1 mM  $\text{Na}_2\text{EDTA}$  and resuspended in 4.5 milliliters of 50 mM Tris, 5 mM EDTA, and 1% SDS. Proteins were precipitated from the cell lysate by 6 M ammonium acetate pH 7.3 and separated from the nucleic acid by centrifugation at 3000 x g. The nucleic acid was recovered from the supernatant by the addition of an equal volume of 100% isopropanol and
- 30    centrifugation at 2000 x g. The dried nucleic acid pellet was hydrated in 10 mM Tris pH 7.6 and 1 mM  $\text{Na}_2\text{EDTA}$  and stored at 4°C.

**EXAMPLE 2****Detection of AKAP10-1 by MassEXTEND™ Assay Detection Methods**

AKAP10-1 is an allele of the AKAP10 gene with a single nucleotide polymorphism at nucleotide number 156277 (based on the sequence of a genomic clone of the AKAP10 gene, GenBank Accession No. AC005730). The single nucleotide polymorphism is a T to C transversion located in the 3' non-translated region of the gene encoding AKAP10. PCR primers were synthesized by OPERON (Alameda, CA) using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 $\mu$ l PCR reaction with 25ng of human genomic DNA obtained from samples as described in Example 1. Each reaction containing IX PCR buffer (Qiagen, Valencia, CA), 200 $\mu$ M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, CA), 4 mM MgCl<sub>2</sub>, and 25 pmol of the forward primer containing the universal primer sequence and the target specific sequence 5'-TCTCAATCATGTGCATTGAGG-3' (SEQ ID NO: 5) 2 pmol of the reverse primer 5'-AGCGGATAACAATTTACACAGGGATCACACAGCCATCAGCAG-3' (SEQ ID NO: 6) and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon 5'-AGCGGATAACAATTTACACAGG-3' (SEQ ID NO: 7). Alternatively, the biotinylated universal primer could be 5'-GGCGCAGCCTCCACG-3' (SEQ ID NO: 16). After an initial round of amplification of the target with the specific forward and reverse primer, the 5' biotinylated universal primer was hybridized and acted as a reverse primer thereby introducing a 3' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon, which dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label each forward primer used in a genotyping. Thermal cycling was performed in 0.2mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, MA) (calculated temperature) with the following cycling parameters: 94°C for 5 min; 45 cycles: 94°C for 20 sec, 56°C for 30 sec, 72°C for 60 sec; 72°C for 3 min.

### Immobilization of DNA

The 50  $\mu$ l PCR reaction was added to 25  $\mu$ l of streptavidin coated magnetic bead (Dynal) prewashed three times and resuspended in 1M  $\text{NH}_4\text{Cl}$ , 0.06M  $\text{NH}_4\text{OH}$ . The PCR amplicons were allowed to bind to the beads for 15 minutes at room temperature. The beads were then collected with a magnet and the supernatant containing unbound DNA was removed. The unbound strand was released from the double stranded amplicons by incubation in 100 mM NaOH and washing of the beads three times with 10 mM Tris pH 8.0.

### Genotyping

- 10 Genotyping was carried out using the MassEXTEND™ assay and MALDI-TOF. The SNP identified at position 156277 of AKAP10 in the GenBank sequence is represented as a T to C transversion. The MassEXTEND™ assay detected the sequence of the complementary strand at the polymorphic position, thus the primer extension product incorporated either a T or a C. The DNA
- 15 coated magnetic beads were resuspended in 26 mM Tris-HCl pH 9.5, 6.5 mM  $\text{MgCl}_2$  and 50 mM each of dTTPs and 50 mM each of ddCTP, ddATP, ddGTP, 2.5U of a thermostable DNA polymerase (Amersham Pharmacia Biotech, Piscataway, NJ) and 20 pmol of a template specific oligonucleotide primer 5'-CTGGCGCCCCACGTGGTCAA-3' (SEQ ID NO: 8) (Operon, Alameda, CA).
- 20 Primer extension occurs with three cycles of oligonucleotide primer was hybridization and extension. The extension products were analyzed after denaturation from the template with 50 mM  $\text{NH}_4\text{Cl}$  and transfer of 150 nl each sample to a silicon chip preloaded with 150 nl of H3PA (3-hydroxy picolinic acid) (Sigma Aldrich, St. Louis, MO) matrix material. The sample material was
- 25 allowed to crystallize and analyzed by MALDI-TOF (Bruker Daltonics, Billerica, MA, PerSeptive, Foster City, CA). The mass of the primer used in the MassEXTEND™ reaction was 5500.6 daltons. The allelic variant results in the addition of ddC to the primer to produce an extension product having a mass of 5773.8 daltons. The predominant allele is extended by the addition of dT and
- 30 ddG to the primer to produce an extension product having a mass of 6101 daltons.

The SNP that is present in AKAP10-1 is a T to C transversion at nucleotide number 156277 of the sequence of a genomic clone of the AKAP10 gene (GenBank Accession No. AC005730) (SEQ ID NO: 17). SEQ ID NO:17 represents the nucleotide sequence of human chromosome 17, which contains  
5 the genomic nucleotide sequence of the human AKAP10 gene at approximately nucleotide 83,580 to nucleotide 156,577. SEQ ID NO: 18 represents the nucleotide sequence of human chromosome 17, which contains the genomic nucleotide sequence of the human AKAP10-1 allele.

The frequency of the AKAP10-1 allelic variant was measured in a  
10 population of age selected healthy individuals. Five hundred fifty-two (552) individuals between the ages of 18-39 years (276 females, 276 males) and 552 individuals between the ages of 60-79 (184 females between the ages of 60-69, 368 males between the age of 60-79) were tested for the presence of the allelic variant localized in the non-translated 3' region of AKAP 10. Differences in the  
15 frequency of this variant with increasing age groups were observed among healthy individuals. Statistical analysis showed that the significance level for differences in the allelic frequency for alleles between the "younger" and the "older" populations was  $p=0.0009$  and for genotypes was  $p=0.003$ . Differences between age groups are significant. For the total population allele  
20 significance is  $p=0.0009$ , and genotype significance is  $p=0.003$ . The young and old populations were in Hardy-Weinberg equilibrium. A preferential change of one particular genotype was not seen.

The polymorphism is localized in the non-translated 3'-region of the gene encoding the human protein kinase A anchoring protein (AKAP10). The gene is  
25 located on chromosome 17. Its structure includes 15 exons and 14 intervening sequences (introns). The encoded protein is responsible for the sub-cellular localization of the cAMP-dependent protein kinase and, therefore, plays a key role in the G-protein mediated receptor-signaling pathway (Huang et al. PNAS (1007) 94:11184-11189). Since its localization is outside the coding region,  
30 this polymorphism is most likely in linkage disequilibrium (LD) with other non-synonymous polymorphisms that could cause amino acid substitutions and subsequently alter the function of the protein.



**EXAMPLE 3****Discovery of AKAP10-5 Allele**

Genomic DNA was isolated from blood (see Example 1) of seventeen (17) individuals with a genotype CC at the AKAP10-1 gene locus and a single  
5 heterozygous individual (CT) (as described in Example 2). A target sequence in the AKAP10-1 gene which encodes the C-terminal PKA binding domain was amplified using the polymerase chain reaction. PCR primers were synthesized by OPERON (Alameda, CA) using phosphoramidite chemistry. Amplification of the AKAP10-1 target sequence was carried out in individual 50 $\mu$ l PCR reaction with  
10 25ng of human genomic DNA templates. Each reaction containing 1 X PCR buffer (Qiagen, Valencia, CA), 200 $\mu$ M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, CA), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing the universal primer sequence and the target specific sequence 5'-TCC CAA AGT GCT GGA ATT AC-3' (SEQ ID NO: 9), 2pmol of the reverse  
15 primer 5'-GTC CAA TAT ATG CAA ACA GTT G-3'(SEQ ID NO:10). Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (MJ Research, Waltham, MA) (calculated temperature) with the following cycling parameters: 94°C for 5 min; 45 cycles; 94°C for 20 sec, 56°C for 30 sec, 72°C for 60 sec; 72°C 3min. After amplification the  
20 amplicons were purified by chromatography (Mo Bio Laboratories (Solana Beach, CA).

The sequence of the 18 amplicons, representing the target region, was determined using a standard Sanger cycle sequencing method with 25 nmol of the PCR amplicon, 3.2  $\mu$ M DNA sequencing primer 5'-CCC ACA GCA GTT AAT  
25 CCT TC-3' (SEQ ID NO:11) and chain terminating dRhodamine labeled 2', 3' dideoxynucleotides (PE Biosystems, Foster City, CA) using the following cycling parameters: 96°C for 15 sec, 25 cycles: 55°C for 15 sec, 60°C for 4 min. The sequencing products were precipitated by 0.3 M NaOAc and ethanol, the precipitate was centrifuged and dried. The pellets were resuspended in  
30 deionized formamide and separated on a on a 5% polyacrylamide gel. The sequence was determined using the "Sequencher" software (Gene Codes, Ann Arbor, MI).

The sequence of all 17 of the amplicons which are homozygous for the AKAP10-1 SNP revealed a polymorphism at nucleotide position 152171 (numbering for GenBank Accession No. AC005730 for AKAP10 genomic clone) with A replaced by G. This SNP can also be designated as located at nucleotide  
5 2073 of a cDNA clone of the wildtype AKAP10 (SEQ ID NO:1) (GenBank Accession No. AF037439). This single nucleotide polymorphism was designated as AKAP10-5 (SEQ ID NO:3) and results in a substitution of a valine for an isoleucine residue at amino acid position 646 (SEQ ID NO:4).

#### EXAMPLE 4

##### 10 PCR Amplification and MassEXTEND™ assay detection of AKAP10-5 in a healthy donor population

A healthy population stratified by age is a very efficient and a universal screening tool for morbidity associated genes by allowing for the detection of changes of allelic frequencies in the young compared to the old population.  
15 Individual samples of this healthy population base can be pooled to further increase the throughput.

Healthy samples were obtained through the blood bank of San Bernardino, CA. Both parents of the blood donors were of Caucasian origin. Practically a healthy subject, when human, is defined as human donor who  
20 passes blood bank criteria to donate blood for eventual use in the general population. These criteria are as follows: free of detectable viral, bacterial, mycoplasma, and parasitic infections; not anemic; and then further selected based upon a questionnaire regarding history. Thus, a healthy population represents an unbiased population of sufficient health to donate blood according  
25 to blood bank criteria, and not further selected for any disease state. Typically such individuals are not taking any medications.

PCR primers were synthesized by OPERON (Alameda, CA) using phosphoramidite chemistry. Amplification of the AKAP10 target sequence was carried out in single 50 $\mu$ l PCR reaction with 100ng- 1 $\mu$ g of pooled human  
30 genomic DNA in a 50 $\mu$ l PCR reaction. Individual DNA concentrations within the pooled samples were present in equal concentration with the final concentration ranging from 1-25ng. Each reaction contained 1X PCR buffer (Qiagen, Valencia,

CA), 200 $\mu$ M dNTPs, 1U Hotstar Taq polymerase (Qiagen, Valencia, CA), 4 mM MgCl<sub>2</sub>, and 25 pmols of the forward primer containing the universal primer sequence and the target specific sequence

5'-AGCGGATAACAATTTACACAGGGAGCTAGCTTGGAAGATTGC-3' (SEQ ID

- 5 NO:12), 2pmol of the reverse primer 5'-GTCCAATATATGCAAACAGTTG-3' (SEQ ID NO: 10) and 10 pmol of a biotinylated universal primer complementary to the 5' end of the PCR amplicon BIO:5'-AGCGGATAACAATTTACACAGG-3' (SEQ ID NO: 7). After an initial round of amplification with the target with the specific forward and reverse primer, the 5' biotinylated universal primer
- 10 hybridized and acted as a forward primer thereby introducing a 5' biotin capture moiety into the molecule. The amplification protocol resulted in a 5'-biotinylated double stranded DNA amplicon and dramatically reduces the cost of high throughput genotyping by eliminating the need to 5' biotin label every forward primer used in a genotyping.

- 15 Thermal cycling was performed in 0.2 mL tubes or 96 well plate using an MJ Research Thermal Cycler (Waltham, MA) (calculated temperature) with the following cycling parameters: 94°C for 5 min; 45 cycles: 94°C for 20 sec, 56°C for 30 sec; 72°C for 60 sec; 72°C for 3 min.

#### Immobilization of DNA

- 20 The 50  $\mu$ l PCR reaction was added to 25 $\mu$ l of streptavidin coated magnetic beads (Dyna, Oslo, Norway) (Lake Success, NY), which were prewashed three times and resuspended in 1 M NH<sub>4</sub>Cl, 0.06 M NH<sub>4</sub>OH. The 5' end of one strand of the double stranded PCR amplicons were allowed to bind to the beads for 15 minutes at room temperature. The beads were then collected
- 25 with a magnet and the supernatant containing unbound DNA was removed. The hybridized but unbound strand was released from the double stranded amplicons by incubation in 100 mM NaOH and washing of the beads three times with 10 mM Tris pH 8.0.

#### Genotyping

- 30 The identity of the nucleotide present at the polymorphic site of AKAP 10-5 was determined by using the MassEXTEND™ assay and MALDI-TOF (see, U.S. Patent No. 6,043,031). The MassEXTEND™ assay is a primer extension

assay that utilizes a primer that hybridizes adjacent to the polymorphic region and which is extended in the presence of one or more ddNTPs. Extension is stopped by the incorporation of a dideoxy nucleotide. At a polymorphic site the different alleles produce different length extension products, which are

5 distinguishable by mass spectrometry.

The MassEXTEND™ assay detected the sequence of the sense strand and resulted in the incorporation of either T or C into the extension product. The DNA coated magnetic beads were suspended in 26 mM Tris-HCl pH 9.5; 6.5 mM, MgCl<sub>2</sub> and 50 mM each of dTTPs and 50 mM each of ddCTP, ddATP,

10 ddGTP, 2.5U of a thermostable DNA polymerase (Amersham Pharmacia Biotech, Piscataway NJ) and 20 pmol of a template specific oligonucleotide primer 5'-ACTGAGCCTGCTGCATAA-3' (SEQ ID NO:15) (Operon) (Alameda, CA). Primer extension occurs with three cycles of oligonucleotide primer hybridization and extension. The extension products were analyzed after denaturation from

15 the template with 50 mM NH<sub>4</sub>Cl and transfer of 150 nl each sample to a silicon chip preloaded with 150 nl of H<sub>3</sub>PA (3-hydroxy picolinic acid) (Sigma Aldrich, St. Louis, MO) matrix material. The sample material was allowed to crystallize and was analyzed by MALDI-TOF (Bruker Daltonics, Billerica, MA, PerSeptive, Foster City, CA). The primer had a mass of 5483.6 daltons. The allelic variant

20 resulted in the addition of a ddC to the primer to produce an extension product having a mass of 5756.8 daltons. The predominant allele resulted in the addition a T and ddG to the primer giving an extension product with a mass of 6101 daltons.

The frequency of the SNP was measured in a population of age selected

25 healthy individuals. 713 individuals under 40 years of age (360 females, 353 males) and 703 individuals over 60 years of age (322 females, 381 males) were tested for the presence of the SNP, AKAP10-5. Results are presented below in Table 3.

30

TABLE 4					
AKAP10-5 (2073V) frequency comparison in 2 age groups					
			< 40	> 60	delta G allele
Female	Alleles	*G	38.6	34.6	4.0

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5			*A	61.4	65.4	
		Genotypes	G	13.9	11.8	2.1
			GA	49.4	45.7	
			A	36.7	42.5	
10	Male	Alleles	*G	41.4	37.0	4.4
			*A	58.6	63.0	
		Genotypes	G	18.4	10.8	7.7
			GA	45.9	52.5	
			A	35.7	36.7	
15						
	Total	Alleles	*G	40.0	35.9	4.1
			*A	60.0	64.1	
		Genotypes	G	16.1	11.2	4.9
			GA	47.7	49.4	
			A	36.2	39.4	

The difference in the allelic and the genotype frequencies between the two age groups, for the total population, are significant,  $p=0.02$ .

20

#### EXAMPLE 5

##### Discovery of AKAP10-6 and AKAP10-7

Genomic DNA isolation, amplification of the target regions and sequencing of amplicons was carried out as in Example 3. Using the sequence of the cDNA for AKAP10, chromosome 17 was BLAST searched to identify the number of exons. Sanger sequencing of the regions around and containing the exons was performed and resulted in the discovery of AKAP10-6 and AKAP10-7 polymorphic regions. For AKAP10-6 the forward sequencing primer was CCCAGGGAGTTA GTTTTGC (SEQ ID NO: 21) and the reverse sequencing primer was GATCCACGGTCCTCAAAGAA (SEQ ID NO: 22). For AKAP10-7 the forward sequencing primer was CACTGCACCCAGCCTTATG (SEQ ID NO: 23)

and the reverse sequencing primer was CTGGGATGTGAAGGAAAGGA (SEQ ID NO: 24).

#### EXAMPLE 6

##### MassEXTEND™ assay detection of AKAP10-6 and AKAP10-7

5           Samples are obtained and amplified as in Example 4.

          The identity of the nucleotide present at the polymorphic site of AKAP 10-6 is determined by using the MassEXTEND™ assay and MALDI-TOF (see, U.S. Patent No. 6,043,031). The MassEXTEND™ assay detected the sequence of the sense strand and resulted in the incorporation of either G or C into the  
10       extension product. Reactions are carried out as in Example 4. The template specific oligonucleotide primer is 5'-GCCGCCATATTATCAACAA-3' (SEQ ID NO: 19) (Operon) (Alameda, CA). The primer has a mass of 5740.8 daltons. The allelic variant results in the addition of a ddC to the primer to produce an  
15       extension product having a mass of 6014.0 daltons. The predominant allele results in the addition of a G and ddC to the primer giving an extension product with a mass of 6343.2 daltons.

          The identity of the nucleotide present at the polymorphic site of AKAP 10-7 is determined by using the MassEXTEND™ assay and MALDI-TOF (see, U.S. Patent No. 6,043,031). The MassEXTEND™ assay detects the sequence of  
20       the complementary strand and resulted in the incorporation of either G or A into the extension product. Reactions are carried out as in Example 4. The template specific oligonucleotide primer is 5'-CTCTGCGTCTCAGGTATT-3' (SEQ ID NO: 20). (Operon, Alameda, CA). The primer has a mass of 5456.6 daltons. The allelic variant results in the addition of a ddA to the primer to produce an  
25       extension product having a mass of 5753.6 daltons. The predominant allele results in the addition of a G and ddA to the primer giving an extension product with a mass of 6083.0 daltons.

          Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

**WHAT IS CLAIMED:**

1. An isolated nucleic acid molecule, comprising a sequence of nucleotides that encodes a polypeptide set forth in SEQ ID No. 2, except that the Ile residue at position 646 of SEQ ID NO: 2 is replaced with Val, Leu or Phe.
- 5 2. An isolated nucleic acid molecule of claim 1, wherein the residue at position 646 of SEQ ID NO: 2 is Val.
3. An isolated nucleic acid molecule of claim 1, comprising the sequence of nucleotides set forth as position 138 to position 2126 of SEQ ID NO: 1, except that the nucleotide at position 2073 of SEQ ID NO: 1 is replaced
- 10 with a nucleotide selected from the group consisting of G, T and C.
4. The nucleic acid molecule of claim 3, wherein the nucleotide at position 2073 of SEQ ID NO: 1 is G.
5. The isolated nucleic acid molecule of claim 2, comprising nucleotides from position 138 to position 2126 of SEQ ID NO: 3.
- 15 6. An isolated nucleic acid molecule, comprising at least 14 or 16 contiguous nucleotides of SEQ. ID. NO: 3; wherein the contiguous nucleotides include a sequence of 5 contiguous nucleotides as set forth from position 2069 to position 2077 of SEQ. ID. NO: 3.
7. The isolated nucleic acid molecule of claim 6, comprising at least
- 20 30 contiguous nucleotides of SEQ. ID. NO: 3.
8. The isolated nucleic acid molecule of claim 6, comprising at least 50 contiguous nucleotides of SEQ. ID. NO: 3.
9. A polypeptide encoded by the nucleic acid molecule of claim 1, comprising at least 5 or 6 amino acid residues including the replaced residue at
- 25 position 646 of SEQ ID NO: 2.
10. The polypeptide of claim 9, wherein the residue at position 646 of SEQ ID NO: 2 is Val.
11. A primer, probe or antisense nucleic acid molecule, comprising a sequence of nucleotides that specifically hybridizes adjacent to or at a
- 30 polymorphic region spanning a position corresponding to position 2073 of SEQ ID No. 1 or 3 of an AKAP10 allele or the complement thereof.

12. The primer, probe or antisense nucleic acid molecule of claim 11, wherein the hybridization refers to hybridization under moderate or high conditions.
13. A primer of claim 11, that specifically hybridizes at a position  
5 immediately adjacent to a position corresponding to position 2073 of SEQ ID NO: 1 or 3 of an AKAP10 allele.
14. A primer of claim 11, that is extended by a nucleotide that specifically base pairs with the nucleotide at a position corresponding to position 2073 of SEQ ID NO: 3 of an AKAP10 allele.
- 10 15. The primer, probe or antisense nucleic acid molecule of claim 11, that is single-stranded and contains at least 14 or 16 contiguous nucleotides of the AKAP10 allele or complement thereof, wherein the sequence of nucleotides includes at least 5 contiguous nucleotides from position 2069 to position 2077 of SEQ. ID. NO: 3.
- 15 16. The primer, probe or antisense nucleic acid molecule of claim 15, wherein nucleotide at position 2073 of SEQ ID NO: 1 is replaced with a nucleotide selected from the group consisting of G, C or T.
17. The isolated nucleic acid molecule of claim 11, comprising at least 20 contiguous nucleotides of SEQ. ID. NO: 3, or the complement thereof.
- 20 18. The isolated nucleic acid of claim 11, comprising at least 30 contiguous nucleotides of SEQ. ID. NO: 3, or the complement thereof.
19. A nucleic acid vector, comprising the nucleic acid molecule of claim 1.
20. A cell containing the nucleic acid vector of claim 19.
- 25 21. A method for detecting the presence or absence of an allelic variant of a human AKAP10 gene, comprising determining the identity of the nucleotide at a position corresponding to position 2073 of the coding sequence of a human AKAP10 gene of claim 1 or the complement thereof, wherein a variant has a nucleotide other than A at a position corresponding to position  
30 2073.



22. The method of claim 21, wherein determining the identity comprises:

- (a) hybridizing a target nucleic acid comprising a human AKAP10-encoding nucleic acid or fragment thereof or a complement of a human AKAP10-encoding nucleic acid or fragment thereof with a nucleic acid primer that hybridizes adjacent to a position corresponding to position 2073 of the coding sequence of the human AKAP10 gene or complement thereof;
  - (b) extending the nucleic acid primer using the target nucleic acid as a template; and
  - (c) determining the mass of the extended primer to identify the nucleotide present at a position corresponding to position 2073 or the complement thereof, thereby determining the presence or absence of an allelic variant.
23. The method of claim 22, wherein the mass is determined by mass spectrometry.
24. The method of claim 22, wherein the primer is extended in the presence of at least one dideoxynucleotide,
25. The method of claim 24, wherein the at least one dideoxynucleotide is ddT.
26. The method of claim 24, wherein the primer is extended in the presence at least two dideoxynucleotides and the at least two dideoxynucleotides are ddT and ddC.
27. The method of claim 26, wherein the mass is determined by mass spectrometry.
28. The method of claim 24, wherein the at least one dideoxynucleotide is ddA.
29. The method of claim 24, wherein the primer is extended in the presence at least two dideoxynucleotides and the at least two dideoxynucleotides are ddA and ddG.
30. The method of claim 29, wherein the mass is determined by mass spectrometry.

31. The method of claim 22, wherein hybridization is effected under conditions of high stringency.

32. The method of claim 21, wherein determining the identity comprises:

5 (a) hybridizing a target nucleic acid comprising a human AKAP10-encoding nucleic acid, complement thereof or fragment thereof with a single-stranded nucleic acid probe at a position corresponding to position 2073 of the coding sequence of the human AKAP10 gene or complement thereof; and

(b) detecting hybridized probe to identify the nucleotide present at a  
10 position corresponding to position 2073 or the complement thereof, thereby determining the presence or absence of an allelic variant.

33. The method of claim 32, wherein hybridization is effected under conditions of high stringency.

34. The method of claim 32, wherein the nucleotide of the probe that  
15 hybridizes with the nucleotide at a position corresponding to position 2073 is complementary to a G, T, or C nucleotide.

35. The method of claim 32, wherein the nucleotide of the probe that hybridizes with the nucleotide at the complement of a position corresponding to position 2073 is complementary to a G, A, or C nucleotide.

20 36. The method of claim 34, wherein the nucleotide detected at the position corresponding to position 2073 is a G.

37. The method of claim 35, wherein the nucleotide detected at the complement of the position corresponding to position 2073 is a C.

25 38. A method for indicating susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality of a subject, comprising:

detecting the presence or absence of at least one allelic variant of a polymorphic region of an AKAP10 gene that is associated with susceptibility to morbidity, increased or early mortality, or morbidity and increased or early  
30 mortality, wherein:

the predominant allele comprises an "A" at a position corresponding to position 2073 of SEQ ID NO: 1; and

the presence of the allelic variant is indicative of increased susceptibility to morbidity, increased or early mortality, or morbidity and increased or early mortality compared to the susceptibility of a subject who does not comprise the allelic variant.

- 5           39. The method of claim 38, wherein a polymorphic region of the AKAP10 gene comprises a nucleotide other than an A at a position corresponding to position 2073 of the coding sequence of the AKAP10 gene or other than a T of the complement of the coding sequence of the AKAP10 gene.
- 10           40. The method of claim 38, wherein the detecting step is effected by a method selected from the group consisting of allele specific hybridization, primer specific extension, oligonucleotide ligation assay, restriction enzyme site analysis and single-stranded conformation polymorphism analysis.
41. The method of claim 38, wherein the detecting step comprises mass spectrometry.
- 15           42. The method of claim 38, wherein detection is effected by detecting a signal moiety selected from the group consisting of radioisotopes, enzymes, antigens, antibodies, spectrophotometric reagents, chemiluminescent reagents, fluorescent reagents and other light producing reagents.
43. The method of claim 39, further comprising:
- 20           detecting an allelic variant at another polymorphic region of an AKAP10 gene selected from the group consisting of a position corresponding to position 83587 of SEQ ID NO: 13, a position corresponding to position 129,600 of SEQ ID NO: 14 and a position corresponding to position 156,277 of SEQ ID NO: 18.
44. A cell, comprising heterologous nucleic acid that encodes a human
- 25           AKAP10 variant protein or portion that exhibits a biological activity of the full length variant protein, wherein the AKAP10 variant protein or portion thereof is encoded by the nucleic acid molecule of claim 1 and comprises valine at a position corresponding to the position of amino acid residue 646 of SEQ ID NO: 2.
- 30           45. The cell of claim 44, wherein heterologous nucleic acid encodes the sequence of amino acids set forth in SEQ. ID. NO: 4.

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46. The cell of claim 44, wherein the nucleic acid comprises the sequence of nucleotides set forth from position 138 to position 2126 of SEQ. ID. NO: 3.

47. A kit for indicating whether a human subject has an increased  
5 susceptibility to morbidity or a predisposition for premature or increased or early mortality, comprising:

a first primer or probe of claim 11; and

a second primer or probe that specifically hybridizes adjacent to or at a polymorphic region spanning a position corresponding to positions selected from  
10 the group consisting of position 83587 of SEQ ID NO 13 or 17, position 129600 of SEQ ID NO 14 or 17, and position 156,277 of SEQ ID NO 18 or 17 of an AKAP10 allele or the complement thereof.

48. The kit of claim 47, further comprising instructions for use.

49. The kit of claim 47, further comprising at least one  
15 dideoxynucleotide.

50. The kit of claim 49, wherein the dideoxynucleotide is selected from the group consisting of ddA, ddC and ddG.

51. A method of producing a protein by growing the cell of claim 20 under conditions whereby the protein is expressed; and  
20 isolating the protein.

52. The method of claim 51, wherein the cell is a mammalian cell, yeast cell, insect cell or bacterial cell.

53. The method of claim 51, wherein the cell is a human cell.

54. A protein produced by the method of claim 52.

25 55. A transgenic animal, comprising heterologous nucleic acid encoding a human AKAP10 variant protein of claim 1 or portion thereof which retains a biological activity exhibited by the full length variant protein, wherein the AKAP10 protein or portion thereof comprises valine at a position corresponding to amino acid residue position 646 of SEQ ID NO: 2, wherein:  
30 the transgenic nucleotide acid is expressed; and,  
as a result of the expression, the transgenic animal has an alteration in cellular signal transduction.

56. The transgenic animal of claim 55, which is a mouse.

57. A method for identifying a molecule that modulates the biological activity of an AKAP10 protein, comprising:

- (a) combining the candidate molecule with a cell comprising a nucleic acid molecule of claim 1 encoding an AKAP10 protein that comprises an amino acid at a position corresponding to the position of residue 646 of SEQ ID NO: 2 which is not Ile or portion thereof that retains a biological activity exhibited by a full length variant protein, operably linked to a promoter such that the nucleotide sequence is expressed as an AKAP protein or portion thereof in the cell; and
- (b) determining the affect of the molecule upon a biological activity of the AKAP10 protein or portion thereof.

58. The method of claim 57, wherein the biological activity of the AKAP protein or portion thereof is determined by examining signal transduction in the cell.

59. The method of claim 57, wherein the biological activity is the binding of AKAP10 protein or portion thereof to protein kinase A.

60. The method of claim 57, wherein the biological activity of the AKAP protein or portion thereof is determined by examining protein phosphorylation in the cell.

61. A method for indicating an alteration in signal transduction in a subject, comprising:

detecting the presence or absence of an allelic variant of an AKAP10 gene having a nucleotide other than A at a position corresponding to position 2073 of SEQ ID NO: 1, wherein the presence of a nucleotide other than A is indicative of an alteration in signal transduction.

62. The method of claim 61, wherein the allelic variant has a G at a position corresponding to position 2073 of SEQ ID NO: 1.

63. The method of claim 61, further comprising detecting the presence or absence of an allelic variant at another polymorphic position of the AKAP10 gene selected from the group consisting of a position corresponding to position 83587 of SEQ ID NO: 13, position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18.

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64. The method of claim 61, wherein the alteration in signal transduction is related to a disorder selected from the group consisting of cardiovascular disorders, cardiac disorders, proliferative disorders, neurological disorders, neurodegenerative disorders, obesity, diabetes and peripheral  
5 retinopathies.

65. The method of claim 64, wherein the disorders are selected from the group consisting of Alzheimer's disease, altered left ventricular function, cardiomyopathies, bipolar disorder and retinitis pigmentosa.

66. The method of claim 61, wherein the detecting step is effected by  
10 a method selected from the group consisting of allele specific hybridization, primer specific extension, oligonucleotide ligation assay, restriction enzyme site analysis and single-stranded conformation polymorphism analysis.

67. The method of claim 61, wherein the detecting step comprises mass spectrometry.

15 68. The method of claim 61, wherein the detecting step is effected a signal moiety selected from the group consisting of: radioisotopes, enzymes, antigens, antibodies, spectrophotometric reagents, chemiluminescent reagents, fluorescent reagents and other light producing reagents.

20 69. A solid support comprising a nucleic acid molecule comprising a polymorphic region of an AKAP10 gene, wherein the polymorphic region comprises a nucleotide at a position corresponding to position 2073 of SEQ ID NO: 1 that is other than an A or other than T on the complementary strand.

70. The solid support of claim 69 which is a microarray.

25 71. The microarray of claim 70, further comprising a nucleic acid molecule that comprises the sequence of a polymorphic region of an AKAP10 gene corresponding to a position selected from the group consisting of position 83587 of SEQ ID NO: 13, position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18.

30 72. An anti-AKAP10 ribozyme comprising a sequence complementary to a polymorphic region of an AKAP10 gene.

73. The ribozyme of claim 72, in which the polymorphic regions are selected from the group consisting of a position corresponding to position 2037

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of SEQ ID NO: 3, position 83587 of SEQ ID NO: 13, position 129,600 of SEQ ID NO: 14 and position 156,277 of SEQ ID NO: 18.

74. The ribozyme of claim 72, comprising SEQ ID NO: 25.

75. A primer consisting essentially of sequence of nucleotides selected  
5 from the group consisting of SEQ ID NO: 8, SEQ ID NO: 15, SEQ ID NO: 19 and  
SEQ ID NO: 20.

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## SEQUENCE LISTING

<110> Braun, A.  
Sequenom, Inc.

<120> POLYMORPHIC KINASE ANCHOR PROTEINS  
AND NUCLEIC ACIDS ENCODING THE SAME

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<309> 1997-12-21

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Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu

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			95					100					105							
aga	tct	tgt	ctg	gac	tac	cag	act	caa	gag	acc	aaa	tca	agc	ctt	tct	506				
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		125				130					135									
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220					225					230					235					
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Leu	Leu	Leu	Ser	Gln	Glu	Cys	Asp	Ser	Ala	His	Ser	Leu	Arg	Leu	Glu					
				240					245					250						
atg	gcc	aga	gca	gga	act	cac	caa	gtt	tcc	atg	gaa	acc	caa	gaa	tct	938				
Met	Ala	Arg	Ala	Gly	Thr	His	Gln	Val	Ser	Met	Glu	Thr	Gln	Glu	Ser					
			255					260					265							
tcc	tct	aca	ctt	aca	gta	gcc	agt	aga	aat	agt	ccc	gct	tct	cca	cta	986				
Ser	Ser	Thr	Leu	Thr	Val	Ala	Ser	Arg	Asn	Ser	Pro	Ala	Ser	Pro	Leu					
		270					275					280								
aaa	gaa	ttg	tca	gga	aaa	cta	atg	aaa	agt	ata	gaa	caa	gat	gca	gtg	1034				
Lys	Glu	Leu	Ser	Gly	Lys	Leu	Met	Lys	Ser	Ile	Glu	Gln	Asp	Ala	Val					
	285					290					295									
aat	act	ttt	acc	aaa	tat	ata	tct	cca	gat	gct	gct	aaa	cca	ata	cca	1082				
Asn	Thr	Phe	Thr	Lys	Tyr	Ile	Ser	Pro	Asp	Ala	Ala	Lys	Pro	Ile	Pro					
300					305					310					315					
att	aca	gaa	gca	atg	aga	aat	gac	atc	ata	gca	agg	att	tgt	gga	gaa	1130				
Ile	Thr	Glu	Ala	Met	Arg	Asn	Asp	Ile	Ile	Ala	Arg	Ile	Cys	Gly	Glu					
				320					325					330						
gat	gga	cag	gtg	gat	ccc	aac	tgt	ttc	gtt	ttg	gca	cag	tcc	ata	gtc	1178				

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Asp Gly Gln Val	Asp Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val	
335	340 345	
ttt agt gca atg gag caa gag cac ttt agt gag ttt ctg cga agt cac		1226
Phe Ser Ala Met Glu Gln Glu His Phe Ser Glu Phe Leu Arg Ser His		
350	355 360	
cat ttc tgt aaa tac cag att gaa gtg ctg acc agt gga act gtt tac		1274
His Phe Cys Lys Tyr Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr		
365	370 375	
ctg gct gac att ctc ttc tgt gag tca gcc ctc ttt tat ttc tct gag		1322
Leu Ala Asp Ile Leu Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu		
380	385 390 395	
tac atg gaa aaa gag gat gca gtg aat atc tta caa ttc tgg ttg gca		1370
Tyr Met Glu Lys Glu Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala		
400	405 410	
gca gat aac ttc cag tct cag ctt gct gcc aaa aag ggg caa tat gat		1418
Ala Asp Asn Phe Gln Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp		
415	420 425	
gga cag gag gca cag aat gat gcc atg att tta tat gac aag tac ttc		1466
Gly Gln Glu Ala Gln Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe		
430	435 440	
tcc ctc caa gcc aca cat cct ctt gga ttt gat gat gtt gta cga tta		1514
Ser Leu Gln Ala Thr His Pro Leu Gly Phe Asp Asp Val Val Arg Leu		
445	450 455	
gaa att gaa tcc aat atc tgc agg gaa ggt ggg cca ctc ccc aac tgt		1562
Glu Ile Glu Ser Asn Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys		
460	465 470 475	
ttc aca act cca tta cgt cag gcc tgg aca acc atg gag aag gtc ttt		1610
Phe Thr Thr Pro Leu Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe		
480	485 490	
ttg cct ggc ttt ctg tcc agc aat ctt tat tat aaa tat ttg aat gat		1658
Leu Pro Gly Phe Leu Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp		
495	500 505	
ctc atc cat tcg gtt cga gga gat gaa ttt ctg ggc ggg aac gtg tcg		1706
Leu Ile His Ser Val Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser		
510	515 520	
ccg act gct cct ggc tct gtt ggc cct cct gat gag tct cac cca ggg		1754
Pro Thr Ala Pro Gly Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly		
525	530 535	
agt tct gac agc tct gcg tct cag tcc agt gtg aaa aaa gcc agt att		1802
Ser Ser Asp Ser Ser Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile		
540	545 550 555	
aaa ata ctg aaa aat ttt gat gaa gcg ata att gtg gat gcg gca agt		1850
Lys Ile Leu Lys Asn Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser		
560	565 570	
ctg gat cca gaa tct tta tat caa cgg aca tat gcc ggg aag atg aca		1898
Leu Asp Pro Glu Ser Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr		
575	580 585	

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ttt gga aga gtg agt gac ttg ggg caa ttc atc cgg gaa tct gag cct	1946
Phe Gly Arg Val Ser Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro	
590 595 600	
gaa cct gat gta agg aaa tca aaa gga tcc atg ttc tca caa gct atg	1994
Glu Pro Asp Val Arg Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met	
605 610 615	
aag aaa tgg gtg caa gga aat act gat gag gcc cag gaa gag cta gct	2042
Lys Lys Trp Val Gln Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala	
620 625 630 635	
tgg aag att gct aaa atg ata gtc agt gac att atg cag cag gct cag	2090
Trp Lys Ile Ala Lys Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln	
640 645 650	
tat gat caa ccg tta gag aaa tct aca aag tta tga ctcaaaaactt	2136
Tyr Asp Gln Pro Leu Glu Lys Ser Thr Lys Leu *	
655 660	
gagataaagg aaatctgctt gtgaaaaata agagaacttt tttcccttgg ttggattcctt	2196
caacacagcc aatgaaaaca gcactatatt tctgatctgt cactgttgtt tccagggaga	2256
gaatggggag acaatcctag gacttcacc ctaatgcagt tacctgtagg gcataattgg	2316
atggcacatg atgtttcaca cagtgaggag tctttaaagg ttaccaa	2363

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 <213> Homo Sapiens

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35 40 45	
Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn His Ala Leu Leu Glu	
50 55 60	
Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala Ile Ser Ala Asn Met	
65 70 75 80	
Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu Lys Lys Gln Pro Ser	
85 90 95	
His Met Glu Ala Ala His Phe Gly Asp Leu Gly Arg Ser Cys Leu Asp	
100 105 110	
Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser Lys Thr Leu Glu Gln	
115 120 125	
Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe Ile Gln Phe Met Glu	
130 135 140	
Leu Arg Arg Met Glu His Leu Val Lys Phe Trp Leu Glu Ala Glu Ser	
145 150 155 160	
Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala His Ser Leu Asn Thr	
165 170 175	
Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser Pro Ser Lys Lys His	
180 185 190	
Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu Asp Lys Arg Leu Glu	
195 200 205	
Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His Ser Glu Gly Ile Asp	
210 215 220	
Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His Leu Leu Leu Ser Gln	
225 230 235 240	
Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu Met Ala Arg Ala Gly	
245 250 255	

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Thr His Gln Val Ser Met Glu Thr Gln Glu Ser Ser Ser Thr Leu Thr
      260      265      270
Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu Lys Glu Leu Ser Gly
      275      280      285
Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val Asn Thr Phe Thr Lys
      290      295      300
Tyr Ile Ser Pro Asp Ala Lys Pro Ile Pro Ile Thr Glu Ala Met
      305      310      315      320
Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu Asp Gly Gln Val Asp
      325      330      335
Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val Phe Ser Ala Met Glu
      340      345      350
Gln Glu His Phe Ser Glu Phe Leu Arg Ser His His Phe Cys Lys Tyr
      355      360      365
Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr Leu Ala Asp Ile Leu
      370      375      380
Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu Tyr Met Glu Lys Glu
      385      390      395      400
Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala Ala Asp Asn Phe Gln
      405      410      415
Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp Gly Gln Glu Ala Gln
      420      425      430
Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe Ser Leu Gln Ala Thr
      435      440      445
His Pro Leu Gly Phe Asp Asp Val Val Arg Leu Glu Ile Glu Ser Asn
      450      455      460
Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys Phe Thr Thr Pro Leu
      465      470      475      480
Arg Gln Ala Trp Thr Thr Met Glu Lys Val Phe Leu Pro Gly Phe Leu
      485      490      495
Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp Leu Ile His Ser Val
      500      505      510
Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser Pro Thr Ala Pro Gly
      515      520      525
Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly Ser Ser Asp Ser Ser
      530      535      540
Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile Lys Ile Leu Lys Asn
      545      550      555      560
Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser Leu Asp Pro Glu Ser
      565      570      575
Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr Phe Gly Arg Val Ser
      580      585      590
Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro Glu Pro Asp Val Arg
      595      600      605
Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met Lys Lys Trp Val Gln
      610      615      620
Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala Trp Lys Ile Ala Lys
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Met Ile Val Ser Asp Ile Met Gln Gln Ala Gln Tyr Asp Gln Pro Leu
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Glu Lys Ser Thr Lys Leu
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 <222> (138) ... (2126)  
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&lt;221&gt; allele

&lt;222&gt; 2073

&lt;223&gt; Single Nucleotide Polymorphism: A to G

&lt;400&gt; 3

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ccactcccgg aagaaggggc ccttttcgcg ctagtgcagc ggcccctctg gacccggaag 120
tccgggcccgg ttgctga atg agg gga gcc ggg ccc tcc ccg cgc cag tcc 170
               Met Arg Gly Ala Gly Pro Ser Pro Arg Gln Ser
                1             5             10

ccc cgc acc ctc cgt ccc gac ccg ggc ccc gcc atg tcc ttc ttc cgg 218
Pro Arg Thr Leu Arg Pro Asp Pro Gly Pro Ala Met Ser Phe Phe Arg
                15             20             25

cgg aaa gtg aaa ggc aaa gaa caa gag aag acc tca gat gtg aag tcc 266
Arg Lys Val Lys Gly Lys Glu Gln Glu Lys Thr Ser Asp Val Lys Ser
                30             35             40

att aaa gct tca ata tcc gta cat tcc cca caa aaa agc act aaa aat 314
Ile Lys Ala Ser Ile Ser Val His Ser Pro Gln Lys Ser Thr Lys Asn
                45             50             55

cat gcc ttg ctg gag gct gca gga cca agt cat gtt gca atc aat gcc 362
His Ala Leu Leu Glu Ala Ala Gly Pro Ser His Val Ala Ile Asn Ala
                60             65             70             75

att tct gcc aac atg gac tcc ttt tca agt agc agg aca gcc aca ctt 410
Ile Ser Ala Asn Met Asp Ser Phe Ser Ser Ser Arg Thr Ala Thr Leu
                80             85             90

aag aag cag cca agc cac atg gag gct gct cat ttt ggt gac ctg ggc 458
Lys Lys Gln Pro Ser His Met Glu Ala Ala His Phe Gly Asp Leu Gly
                95             100             105

aga tct tgt ctg gac tac cag act caa gag acc aaa tca agc ctt tct 506
Arg Ser Cys Leu Asp Tyr Gln Thr Gln Glu Thr Lys Ser Ser Leu Ser
                110             115             120

aag acc ctt gaa caa gtc ttg cac gac act att gtc ctc cct tac ttc 554
Lys Thr Leu Glu Gln Val Leu His Asp Thr Ile Val Leu Pro Tyr Phe
                125             130             135

att caa ttc atg gaa ctt cgg cga atg gag cat ttg gtg aaa ttt tgg 602
Ile Gln Phe Met Glu Leu Arg Arg Met Glu His Leu Val Lys Phe Trp
                140             145             150             155

tta gag gct gaa agt ttt cat tca aca act tgg tcg cga ata aga gca 650
Leu Glu Ala Glu Ser Phe His Ser Thr Thr Trp Ser Arg Ile Arg Ala
                160             165             170

cac agt cta aac aca atg aag cag agc tca ctg gct gag cct gtc tct 698
His Ser Leu Asn Thr Met Lys Gln Ser Ser Leu Ala Glu Pro Val Ser
                175             180             185

cca tct aaa aag cat gaa act aca gcg tct ttt tta act gat tct ctt 746
Pro Ser Lys Lys His Glu Thr Thr Ala Ser Phe Leu Thr Asp Ser Leu
                190             195             200

gat aag aga ttg gag gat tct ggc tca gca cag ttg ttt atg act cat 794
Asp Lys Arg Leu Glu Asp Ser Gly Ser Ala Gln Leu Phe Met Thr His
                205             210             215

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tca gaa gga att gac ctg aat aat aga act aac agc act cag aat cac Ser Glu Gly Ile Asp Leu Asn Asn Arg Thr Asn Ser Thr Gln Asn His 220 225 230 235	842
ttg ctg ctt tcc cag gaa tgt gac agt gcc cat tct ctc cgt ctt gaa Leu Leu Leu Ser Gln Glu Cys Asp Ser Ala His Ser Leu Arg Leu Glu 240 245 250	890
atg gcc aga gca gga act cac caa gtt tcc atg gaa acc caa gaa tct Met Ala Arg Ala Gly Thr His Gln Val Ser Met Glu Thr Gln Glu Ser 255 260 265	938
tcc tct aca ctt aca gta gcc agt aga aat agt ccc gct tct cca cta Ser Ser Thr Leu Thr Val Ala Ser Arg Asn Ser Pro Ala Ser Pro Leu 270 275 280	986
aaa gaa ttg tca gga aaa cta atg aaa agt ata gaa caa gat gca gtg Lys Glu Leu Ser Gly Lys Leu Met Lys Ser Ile Glu Gln Asp Ala Val 285 290 295	1034
aat act ttt acc aaa tat ata tct cca gat gct gct aaa cca ata cca Asn Thr Phe Thr Lys Tyr Ile Ser Pro Asp Ala Ala Lys Pro Ile Pro 300 305 310 315	1082
att aca gaa gca atg aga aat gac atc ata gca agg att tgt gga gaa Ile Thr Glu Ala Met Arg Asn Asp Ile Ile Ala Arg Ile Cys Gly Glu 320 325 330	1130
gat gga cag gtg gat ccc aac tgt ttc gtt ttg gca cag tcc ata gtc Asp Gly Gln Val Asp Pro Asn Cys Phe Val Leu Ala Gln Ser Ile Val 335 340 345	1178
ttt agt gca atg gag caa gag cac ttt agt gag ttt ctg cga agt cac Phe Ser Ala Met Glu Gln Glu His Phe Ser Glu Phe Leu Arg Ser His 350 355 360	1226
cat ttc tgt aaa tac cag att gaa gtg ctg acc agt gga act gtt tac His Phe Cys Lys Tyr Gln Ile Glu Val Leu Thr Ser Gly Thr Val Tyr 365 370 375	1274
ctg gct gac att ctc ttc tgt gag tca gcc ctc ttt tat ttc tct gag Leu Ala Asp Ile Leu Phe Cys Glu Ser Ala Leu Phe Tyr Phe Ser Glu 380 385 390 395	1322
tac atg gaa aaa gag gat gca gtg aat atc tta caa ttc tgg ttg gca Tyr Met Glu Lys Glu Asp Ala Val Asn Ile Leu Gln Phe Trp Leu Ala 400 405 410	1370
gca gat aac ttc cag tct cag ctt gct gcc aaa aag ggg caa tat gat Ala Asp Asn Phe Gln Ser Gln Leu Ala Ala Lys Lys Gly Gln Tyr Asp 415 420 425	1418
gga cag gag gca cag aat gat gcc atg att tta tat gac aag tac ttc Gly Gln Glu Ala Gln Asn Asp Ala Met Ile Leu Tyr Asp Lys Tyr Phe 430 435 440	1466
tcc ctc caa gcc aca cat cct ctt gga ttt gat gat gtt gta cga tta Ser Leu Gln Ala Thr His Pro Leu Gly Phe Asp Asp Val Val Arg Leu 445 450 455	1514
gaa att gaa tcc aat atc tgc agg gaa ggt ggg cca ctc ccc aac tgt Glu Ile Glu Ser Asn Ile Cys Arg Glu Gly Gly Pro Leu Pro Asn Cys 460 465 470 475	1562

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Leu Pro Gly Phe Leu Ser Ser Asn Leu Tyr Tyr Lys Tyr Leu Asn Asp	
495 500 505	
ctc atc cat tcg gtt cga gga gat gaa ttt ctg ggc ggg aac gtg tcg	1706
Leu Ile His Ser Val Arg Gly Asp Glu Phe Leu Gly Gly Asn Val Ser	
510 515 520	
ccg act gct cct ggc tct gtt ggc cct cct gat gag tct cac cca ggg	1754
Pro Thr Ala Pro Gly Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly	
525 530 535	
agt tct gac agc tct gcg tct cag tcc agt gtg aaa aaa gcc agt att	1802
Ser Ser Asp Ser Ser Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile	
540 545 550 555	
aaa ata ctg aaa aat ttt gat gaa gcg ata att gtg gat gcg gca agt	1850
Lys Ile Leu Lys Asn Phe Asp Glu Ala Ile Val Asp Ala Ala Ser	
560 565 570	
ctg gat cca gaa tct tta tat caa cgg aca tat gcc ggg aag atg aca	1898
Leu Asp Pro Glu Ser Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr	
575 580 585	
ttt gga aga gtg agt gac ttg ggg caa ttc atc cgg gaa tct gag cct	1946
Phe Gly Arg Val Ser Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro	
590 595 600	
gaa cct gat gta agg aaa tca aaa gga tcc atg ttc tca caa gct atg	1994
Glu Pro Asp Val Arg Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met	
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aag aaa tgg gtg caa gga aat act gat gag gcc cag gaa gag cta gct	2042
Lys Lys Trp Val Gln Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala	
620 625 630 635	
tgg aag att gct aaa atg ata gtc agt gac gtt atg cag cag gct cag	2090
Trp Lys Ile Ala Lys Met Ile Val Ser Asp Val Met Gln Gln Ala Gln	
640 645 650	
tat gat caa ccg tta gag aaa tct aca aag tta tga ctcaaaactt	2136
Tyr Asp Gln Pro Leu Glu Lys Ser Thr Lys Leu *	
655 660	
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Lys	Glu	Gln	Glu	Lys	Thr	Ser	Asp	Val	Lys	Ser	Ile	Lys	Ala	Ser	Ile
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Ser	Val	His	Ser	Pro	Gln	Lys	Ser	Thr	Lys	Asn	His	Lys	Ala	Leu	Glu
	50					55					60				
Ala	Ala	Gly	Pro	Ser	His	Val	Ala	Ile	Asn	Ala	Ile	Ser	Ala	Asn	Met
65					70				75					80	
Asp	Ser	Phe	Ser	Ser	Ser	Arg	Thr	Ala	Thr	Leu	Lys	Lys	Gln	Pro	Ser
				85					90					95	
His	Met	Glu	Ala	Ala	His	Phe	Gly	Asp	Leu	Gly	Arg	Ser	Cys	Leu	Asp
			100					105					110		
Tyr	Gln	Thr	Gln	Glu	Thr	Lys	Ser	Ser	Leu	Ser	Lys	Thr	Leu	Glu	Gln
		115					120					125			
Val	Leu	His	Asp	Thr	Ile	Val	Leu	Pro	Tyr	Phe	Ile	Gln	Phe	Met	Glu
	130					135					140				
Leu	Arg	Arg	Met	Glu	His	Leu	Val	Lys	Phe	Trp	Leu	Glu	Ala	Glu	Ser
145					150				155					160	
Phe	His	Ser	Thr	Thr	Trp	Ser	Arg	Ile	Arg	Ala	His	Ser	Leu	Asn	Thr
				165					170					175	
Met	Lys	Gln	Ser	Ser	Leu	Ala	Glu	Pro	Val	Ser	Pro	Ser	Lys	Lys	His
			180					185				190			
Glu	Thr	Thr	Ala	Ser	Phe	Leu	Thr	Asp	Ser	Leu	Asp	Lys	Arg	Leu	Glu
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Asp	Ser	Gly	Ser	Ala	Gln	Leu	Phe	Met	Thr	His	Ser	Glu	Gly	Ile	Asp
	210				215						220				
Leu	Asn	Asn	Arg	Thr	Asn	Ser	Thr	Gln	Asn	His	Leu	Leu	Leu	Ser	Gln
225					230				235					240	
Glu	Cys	Asp	Ser	Ala	His	Ser	Leu	Arg	Leu	Glu	Met	Ala	Arg	Ala	Gly
				245					250				255		
Thr	His	Gln	Val	Ser	Met	Glu	Thr	Gln	Glu	Ser	Ser	Ser	Thr	Leu	Thr
			260					265					270		
Val	Ala	Ser	Arg	Asn	Ser	Pro	Ala	Ser	Pro	Leu	Lys	Glu	Leu	Ser	Gly
		275					280					285			
Lys	Leu	Met	Lys	Ser	Ile	Glu	Gln	Asp	Ala	Val	Asn	Thr	Phe	Thr	Lys
	290				295						300				
Tyr	Ile	Ser	Pro	Asp	Ala	Ala	Lys	Pro	Ile	Pro	Ile	Thr	Glu	Ala	Met
305				310					315					320	
Arg	Asn	Asp	Ile	Ile	Ala	Arg	Ile	Cys	Gly	Glu	Asp	Gly	Gln	Val	Asp
			325						330				335		
Pro	Asn	Cys	Phe	Val	Leu	Ala	Gln	Ser	Ile	Val	Phe	Ser	Ala	Met	Glu
			340					345					350		
Gln	Glu	His	Phe	Ser	Glu	Phe	Leu	Arg	Ser	His	His	Phe	Cys	Lys	Tyr
		355					360					365			
Gln	Ile	Glu	Val	Leu	Thr	Ser	Gly	Thr	Val	Tyr	Leu	Ala	Asp	Ile	Leu
	370					375		</							



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Ser Val Gly Pro Pro Asp Glu Ser His Pro Gly Ser Ser Asp Ser Ser  
           530                                  535                                  540  
 Ala Ser Gln Ser Ser Val Lys Lys Ala Ser Ile Lys Ile Leu Lys Asn  
 545                                  550                                  555                                  560  
 Phe Asp Glu Ala Ile Ile Val Asp Ala Ala Ser Leu Asp Pro Glu Ser  
                                   565                                  570                                  575  
 Leu Tyr Gln Arg Thr Tyr Ala Gly Lys Met Thr Phe Gly Arg Val Ser  
                                   580                                  585                                  590  
 Asp Leu Gly Gln Phe Ile Arg Glu Ser Glu Pro Glu Pro Asp Val Arg  
                                   595                                  600                                  605  
 Lys Ser Lys Gly Ser Met Phe Ser Gln Ala Met Lys Lys Trp Val Gln  
                                   610                                  615                                  620  
 Gly Asn Thr Asp Glu Ala Gln Glu Glu Leu Ala Trp Lys Ile Ala Lys  
 625                                  630                                  635                                  640  
 Met Ile Val Ser Asp Val Met Gln Gln Ala Gln Tyr Asp Gln Pro Leu  
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 Glu Lys Ser Thr Lys Leu  
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21

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&lt;220&gt;

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